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SCHOOL OF CHEMICAL AND BIO-ENGINEERING

Acid hydrolysis of water hyacinth (emboch) for the production of glucose (fermentable sugar)

M.Sc. Thesis in Process Engineering Stream

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A Thesis

Submitted in partial fulfilment of the requirements for the Degree of Master of Science

Dilute Acid Hydrolysis of Water Hyacinth (Emboch) For the Production of Glucose (fermentable sugar)

Tewodros Taddese

A thesis submitted to
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This is to certify that the thesis prepared by Tewodros Taddese, entitled: Dilute acid hydrolysis of water hyacinth (Emboch) for the production of Glucose (Fermentable sugar), submitted in partial fulfillment of the requirements for the degree of masters of science (Chemical and Bio Engineering) complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

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Abstract:

This study investigates production of glucose (fermentable sugar) from water hyacinth pretreated with 1.5% H_2O_2 and 3%NaOH. Cellulose-hemicellulose and lignin content of water hyacinth were determined using thermo gravimetric analysis (TGA) and found to be 42.1% and 11.46% before pretreatment and its crystallinity was measured by X- ray diffraction (XRD) which was 31.29%.The pretreated sample (54.11% cellulose-hemicellulose and 7.04% lignin with 53.68% crystallinity) was hydrolyzed in an autoclave using diluted sulfuric acid (0.5% -1.5%), at a temperature between 100 – 130 ^{o}C for a hydrolysis time between 45 – 75 min. The Highest glucose yield (indicating efficient hydrolysis of cellulose) was found to be 0.451 mg/ml at an acid concentration of 0.5%, temperature of 130 ^{o}C and hydrolysis time of 75 min. In spite of its detrimental effect on environment and society, it is also possible to produce valuable products from the weed. However, further researches should be done on utilization of water hyacinth in small and large scale.

Key words: Water hyacinth, Characterization, Pretreatment, Hydrolysis

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List of Acronyms

AME Africa and the Middle East

BOD Biological Oxygen Demand

COD Chemical Oxygen Demand

DO Dissolved Oxygen

DTG Differential Thermo Gravimeter

EBI Ethiopian Biodiversity Institute

EC Ethiopian Calendar

FSF Fincha Sugar Factory

GC Gregorian Calendar

MSF Metehara Sugar Factory

SHF Separate Hydrolysis and Fermentation

SSF Simultaneous Saccharification and Fermentation

TGA Thermo Gravimetric Analysis

TKN Total Kjeldahl Nitrogen

TSS Total Suspended Solid

UV Ultraviolet

WH Water Hyacinth

WSSF Wonji Shoa Sugar Factory

XRD X-ray diffraction

1. INTRODUCTION

1.1. Background

Water hyacinth, Eichhornia crassipes, is a tropical species belonging to the pickerelweed family (pontederiaceae). It occurs in different water bodies such as lakes, slowly moving rivers and swamps of various countries which are located between 40^o N and 40^o S. This weed is a native of Brazil and probably other central South American countries. It is best known for two things, its reproduction ability and removal of pollutants from water. It only needs short period of time to cover a specific area densely (over 60 kg/m^2).

Although water hyacinth prefers wetland and nutrient-enriched water, it can also survive when there is significant fluctuation in nutrient, temperature and pH levels. In the pH range of 6 – 8 the growth is very fast, and 25 – 27.5 ^oC is the optimum temperature for growth of this weed. In spite of that, it also grows in a wide range of temperature of 1 up to 40 ^oC. As the nitrogen amount in the water rises the growth of water hyacinth increases. On the other hand, Salinity is a major constraint on water hyacinth growth. In coastal regions as salinity levels at 6 and 8% are lethal. Since it appears as a dense mat it affects other living organisms by blocking light and by reducing dissolved oxygen level. It also antagonizes the growth of other organisms by releasing allelochemicals and reduces biodiversity.

It reproduces both asexually through stolon and sexually through seeds, which remain viable for up to 20 years. The weed grows with a tremendous speed (in every 5 – 15 days it doubles the population) and also grows from a few centimeters to about a meter in height. The leaves and stems are air filled sacs, which enables the plant to float in water bodies.

The history of water hyacinth in Ethiopia started from 1956, during this year the appearance of the weed is reported in koka lake and the Awash river. In 1965, it has recognized as a treat causing weed that can damage a lot of water bodies. The species is now present in the lowlands of ilubabor (baro, Gillo and Pibor rivers), in the lake Tana area (marshy tracts of the Blue Nile or Abbay river near its outlet with lake Tana), and in the Rift Valleys (Awash river, Koka dam, lake Ziway and lake Ellen). The largest lake in Ethiopia, is Lake Tana which holds 50% of the country’s fresh water. It is also the main water provider for Blue Nile, which holds up to 60% of the Nile’s water. There is a variety of fish in this lake, which is a food and income source for the community around the lake. Unfortunately, this lake is highly infested with water hyacinth since 2011. In 2011 about 20,000 hectares of the North-eastern part of the lake was infested. After three years the water hyacinth takes one-third of the lake's shoreline, which is around 128 km. After various eradication measures using human power and mechanical machineries, the weed is now estimated to cover 3,000 hectares of the lake. To make matters worse, inflowing rivers carry heavy loads of soil and suspended sediment into the lake, which affects the water quality and creates favorable conditions for the spread of the weed.

Several methods are being applied to remove or control the growth of water hyacinth in different water bodies. The methods can be classified as chemical, biological and mechanical. Chemical control methods can be done by using different herbicides to control the weed. However, this method might cause several problems such as the adverse effect on the environment and public health. The biological control methods have been carried using some pests as fungal pathogens, mites, snails or insects and certain grass-eating fish species, however, the results were not satisfactory. In addition, introducing new pests to the environment may disturb the natural balance and the biodiversity. On the other hand, the mechanical control method is the recommended one since it works by using different machinery devices (e.g. mechanical mooring and rolling machines, conveyers…. etc.).

Recently, attention is being devoted to the utilization of water hyacinth since the efforts to control plant growth by various mechanisms have met with little success. The plans are not to cultivate the plant, since it is considered as a pest, but it is about how to get benefit from the weed. The most important consideration in the utilization of water hyacinth is its high growth rate and dry matter production, therefore the harvesting rate should be equal to or more than the growth rate. Water hyacinth can be utilized as animal feed (non-ruminants-pigs and rabbits-can better utilize the weed than cows and goats), organic fertilizer (green manure and compost) or mulching materials, biogas and ethanol production and phytoremediation.

Therefore, the aim of this paper is to study the process and find the optimum condition to produce simple sugar (glucose)-which can be fermented to produce ethanol- from water hyacinth by using sulfuric acid as a hydrolyzing agent. Currently, molasses-byproduct of the sugar industries-is the main source of fermentable sugars for the alcohol and liquor industries in Ethiopia. Their production capacity is tabulated below.

Table 1: Amount of molasses produced in Ethiopia for the last 6 consecutive years

Milling season		Molasses production (Quintals)						
EC	GC	WSSF	FSF	MSF	Arijo	Kessem	Tendaho	Omo Kuraz 2
2006	2013/14	238,584.68	400,845.2	404,442.47				
2007	2014/15	365,843	551,460	380,011.1				
2008	2015/16	340,553.6	423,580	23,806.6	10,179.6	16,664.5	152,273	
2009	2016/17	299,990	361,525	470,031	51,471	324,382.5		
2010	2017/18	223,450	392,283	256,462.8	29,572	223,700	82,164.8	55,970
2011	2018/19	194,070	354,180	244,520	30,600	190,270		

These sugar factories carry the burden of producing molasses and ethanol for the countries demand. One thing that we should not forget is the priority is to produce sugar not molasses or ethanol. As a result of this, finding another supporting resource for glucose production is a must for our country in order to have sustainable production of alcohols. Therefore, this paper could add value on ongoing researches that are being done on utilization of biomass to produce simple sugar that can be changed to different products such as ethanol.

1.2. Statement of the problem

Nowadays, water hyacinth has been considered as one of the world's most invasive aquatic weed causing problems on most of the community who are dependent on water bodies. It is known as "blue devil" or "Bengal terror" in India, "Florida devil" in South Africa, "German weed" in Bangladesh and "water terror" by South Western Nigeria because of its adverse impact on aquatic ecosystem, agriculture, fisheries, transportation, living condition and social structure. Its labeled as one of the worst weed because of its ability to rapidly cover whole waterways (*Asmare*, *2017*).

In Ethiopia, water hyacinth is called "emboch". It is found in many water bodies of the country which have several uses as a means of transportation, fishery, irrigation and recreation. It forms dense, interlocking mat that covers water bodies and facilitate evapotranspiration. Currently, the total coverage of the weed reached 10,647 hectares. However, this data represents only the northern and eastern part of the country, the western and southern parts have not yet reported the areas which are infested with this weed.

In this thesis, simple sugar (glucose) production from water hyacinth will be considered as an alternative for local fermentable sugar production with an emphasis on optimization of the hydrolysis process. There are several types of waste materials that can potentially be used for glucose production, but these seaweeds are causing a great problem at the Lake Tana region. It is very invasive, and peoples are trying to remove it by mechanical means. Different researches said that complete eradication of the weed might be unimaginable. So, we should at least get some benefit from this weed despite of its socioeconomic and ecological detriment.

1.3. Objectives of the Research

1.3.1. General Objective

The general objective of the study is to optimize the hydrolysis process for glucose production from water hyacinth - extremely invasive seaweed - using dilute acid hydrolysis.

1.3.2. Specific Objectives

- To measure the effect of pretreatment on water hyacinth.
- To produce glucose
- To study the effect of acid concentration, temperature and time on the hydrolysis process.
- To find the optimum hydrolyzing parameters to produce fermentable sugar (glucose).

1.4. Significance of the Research

This study should be significant in the sense that it might:

- Provide alternative raw material for fermentable sugar production.
- Investigate the potential of water hyacinth to produce glucose.
- Provide useful knowledge on factors that might have impact on the hydrolyzation process.
- Play a role in research and development efforts to reduce disposal cost of collected WH

2. LITERATURE REVIEW

2.1 water hyacinth infestation around the world

Water hyacinth (Eichhornia crassipes) has been listed as one of the most productive plant on earth which has invaded freshwater system in over 50 countries on five continents, especially throughout southeast Asia, the south eastern united states, central and western Africa and central America. It is efficient in using nutrients from water bodies and solar energy for copious biomass production, bringing environmental, social and economic problems. This weed can be found in lakes and estuaries, wetlands, marshes, ponds, dams, slow flowing rivers, streams and waterways in lower latitudes in which its growth is facilitated because of the inflow of nutrients rich water from urban and agricultural runoff, deforestation, products of industrial waste and insufficient wastewater treatment. There is also a chance of distribution of water hyacinth into higher latitudes due to rise of temperature in these areas (*Bhattacharya, Haldar, & Chatterjee, 2015*).

It has spread throughout vital freshwater bodies and wetlands of Africa and the middle east (AME) since the late 1800s. based on the survey that was done on 29 AME countries, most of these countries have water hyacinth, and 21 of them confirmed the weed is already a problem within their borders (*Navarro & George, 2000*).

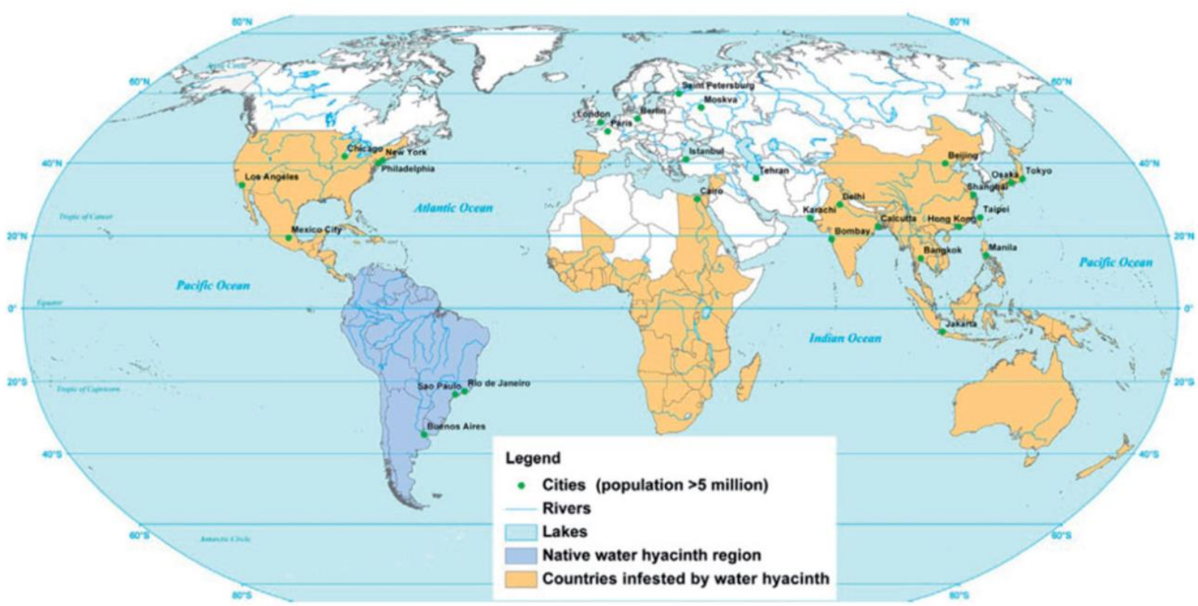

Figure 2: Global distribution of water hyacinth (*S. H. Yan, Song, & Guo, 2017*)

Its distribution is mainly limited by its winter-survival temperature at around 7 ^oC, although a short period of exposure at 5 ^oC may not entirely destroy the macrophyte and has also extended into further north and south lattitudes. The minimum temperature requirement to start growing is around 10 ^oC and up to 43 ^oC the plant can survive (*S. H. Yan et al., 2017*).

2.2 water hyacinth infestation in Ethiopia

In Ethiopia, water hyacinth was officially reported in 1956 in Koka Lake and the Awash River (Stroud, 1994). The infestation was very small by the time and the appropriate authorities had not taken an action even if they were notified. In 1962, the weed had succeeded in infesting the the whole stretches of the White Nile from Juba to Jebel Aulia Dam; the whole length of the Sobat River from its mouth eastward up to Baro and Gillo rivers in Ethiopia.

During the peak rainy season's water hyacinth infestation became facilitated among diverse and connected water bodies. The weed started to infest Awash River possible through Abasamuel dam that is organically enriched by Akaki River, in which a lot of urban wastes of Addis Ababa is disposed. It is believed that the flood of Awash River, in 1996, around the factory of Wonji-Shoa introduced the weed from Koka Hydroelectric Power Dam.

Based on the survey carried out by (*Tegene & Ayele, 2014*) in some of the Awash Rivers catchments including Abasamuel Dam, Koka Dam, Lake Ellen, Wonji-Shoa Estate and Awash river near Koka town about 75% of the surveyed water bodies had more than 50% infestation level. What makes matters worse is 50 % of the water bodies had more than 87% infestation.

One of the largest lakes in Ethiopia is Lake Tana. Its infestation with water hyacinth is not exactly known but based on unstructured interviews to the local fishers and communities, 50 % have said that it was detected in 2009 and the rest responded as it has only observed since July, 2011(D Tewabe, 2015). In 2012, water hyacinth had proliferated and covered about 15% of the northern shore of the lake. Even if several removal campaigns were organized by the regional government, it kept flourishing alarmingly year to year. It was estimated that its infestation coverage had reached 34,500 ha (3,000 ha thick, 2,500 ha intermediate and 29,000 ha scattered) in 2015 (*Asmare, 2017*).

According to the unpublished research of Ethiopian Biodiversity Institute, current water hyacinth infestation dropped down to around 3,000 ha along all woredas around Lake Tana. However, this report doesn't include those water bodies infested by the weed in other part of the country. On the other hand, around 10,000 ha of Lake Chamo is overran by water hyacinth.

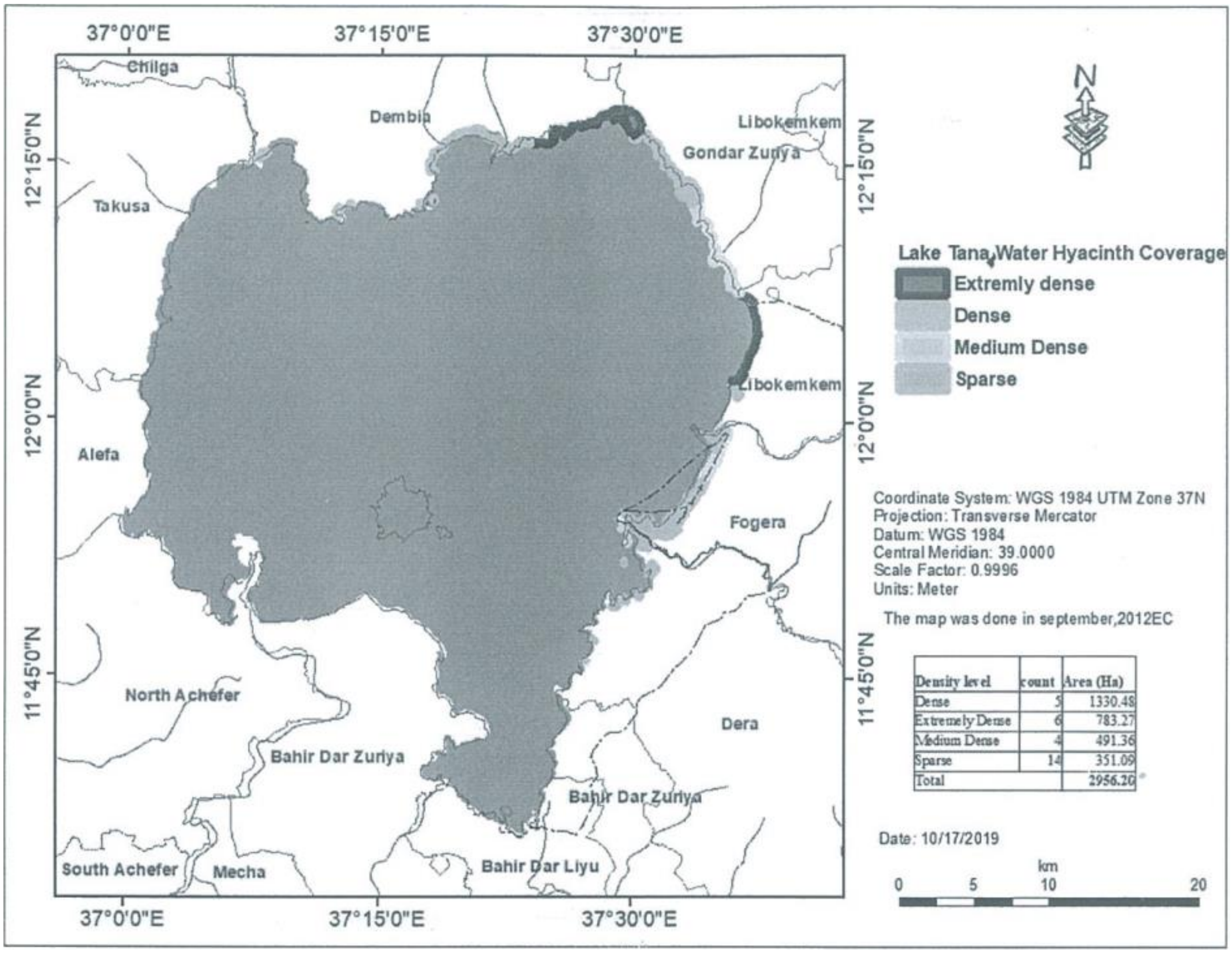

Figure 2.1: water hyacinth area coverage of Lake Tana, October 2019 (*EBI*)

2.3 Challenges posed by water hyacinth

Invasions of water hyacinth vary in extent and duration from place to place, but generally it causes similar problems. The change in its density have the potential to affect other ecological and human communities in areas where it is established (*Villamagna & Murphy, 2010*). A few researchers have tried to study the impact of this invasive weed on ecological communities, environment and human health.

2.3.1 Impact of water hyacinth on ecological communities

The structure of a macrophyte community plays a large role in determining community composition of phytoplankton, zooplankton and fish in freshwater ecosystems (*Villamagna & Murphy, 2010*). In texas, (*Madsen, 1997*) found that hyacinth had the lowest dissolved oxygen level as compared to milfoil, hydrilla, pondweed and a native mix of submerged plants, and was the only plant to have average below 5 mg^{-1}. At this dissolved oxygen level many fishes start to experience oxygen stress.

Free floating plants (such as water hyacinth) can dominate phytoplankton and submersed vegetation through monopolizing light and absorb nutrients from water bodies, preventing them from obtaining sufficient resources for photosynthesis (*Scheffer et al., 2003*).

Based on the research of (*Sciences, 2003*) which was done on selected sites with water hyacinth, they could not get epibenthic amphipods and isopods beneath the hyacinth canopy. This is likely the result of lower level of dissolved oxygen. On the other hand, an abundance of amphipods and isopods were living epiphytically amongst the roots that suggests that these invertebrates could potentially be taking refuges in the root mass from underlying hypoxia. Its effect was compared with pennywort at the same site which did have both epibenthically underneath the canopies and epiphytically among the roots. The density of invertebrates was greater in pennywort than hyacinth.

2.3.2 Social and economic impact

The impact of water hyacinth on social and economy is visible on areas where its infestation is reported. This weed poses direct or indirect problems for many human uses. Problems on boating, water intake pipes for agriculture and industry, navigation and recreation are amongst of the direct impacts. Furthermore, it increases cost of water treatment, affects fish catchability and increases evapotranspiration. On the other hand, it also impact wildlife and ecosystem service (*Villamagna & Murphy, 2010*).

Local people who lives on the lakeside of Kenya, Tanzania and Uganda responded that there was a decrease in fish catches, increased transportation costs, and difficulties associated with clean water availability as major negative impacts (*Mailu, 2000*).

1. *Fish catchability*

(*Mailu, 2000*) reported that there was a decrease on the amount of specific fish species (Oreochromis, Clarias and Mormyrus) caught during 1991-1997. There was a 14, 37 and 59 % decrease in Oreochromis, Clarias and Mormyrus fish species respectively. In addition to that, hinderance of land and water accessibility resulted reduction of fish catches, especially those which are found mainly along the shores (Tilapia and mudfish). On another study which was undertaken by (Kateregga & Sterner, 2009) there was a reduction on the amount of fish caught in Kenya, Tanzania and Uganda section of lake Victoria between 1993 to 2000. The percentage decrease in these three countries were 45, 2 and 6 % respectively. In Ethiopia, the study of (*Asmare, 2017*), indicates that fishers of Nabega Kebele around lake tana changed their landing site to their nearest one Libo-kemkem to get rid of water hyacinth interference on their activity. (*Dereje Tewabe, Asmare, Zelalem, & Mohamed, 2017*) also reported that fishing became difficult on Lake Tana as it requires disengagement of water hyacinth from gillnet after catching. In the process, the material might be damaged and incur maintenance cost on the fishers.

2. *Transportation*

In 1997, water hyacinth coverage in Florida reached as many as 125,000 acres with a mass of 200 tons per acre resulting temporary closing of boat traffics in several rivers (*Malik, 2007*). In Kenya, both cargo and human transportation through small vessels were hindered because of the infestation and also caused temporary shutdown of piers in some areas (*Mailu, 2000*). In Ethiopia, Aba Samuel dam was one of the artificial lakes affected by water hyacinth. Recreational activities such as boating and sailing were affected because of the weed (*Ingwani, Gumbo, & Gondo, 2010*).

3. *Water quality and other effects*

In lake Tana, where water hyacinth infestation is significant, the amount of dissolved solid and conductivity increased whereas pH and dissolved oxygen concertation became very low (*Gezie et al., 2018*). It was also reported by Kenya and Uganda that water hyacinth affects turbidity, color and pH of water (*Mailu, 2000*).

Among various problems of water hyacinth, blockage of intake pipe is also the main issue. There was a financial lose and 25 % reduction of water to the Kisumu, Kenya, municipal council water supply in 1999, because of intake blockage with water hyacinth (*Opande, Onyango, & Wagai*, *2004*). Water loss due evapotranspiration is the other problem. Based on the research conducted in Roseta branch of Nile river, by Ali and Kedir, the average water loss through evapotranspiration of the weed was estimated to be 21.3 million m^3/year (*Merry M. Mitan, 2019*).

In addition to the abovementioned ones, it also affects cattle and their owners indirectly. Expansion of water hyacinth in Lake Tana destroyed many native aquatic plants. One of those is submerging grass (hippo grass), which is a food source for the surrounding cattle. As a result of this, the community is forced to buy animal feeds from the market (*Dereje Tewabe et al., 2017*).

2.3.3 Effect on human health

The amount of dissolved oxygen is very low under areas which are infested by water hyacinth. This inturn creats a very good enviroment for breeding of mosquito vectors of malaria, encephalities and filariasis. Lake victoriya was one of the tropical lake, in which breeding of snails, mosquitos and snakes were detected because of the weed (*Malik*, *2007*).

A research on Aba samuel dam (which is found in Ethiopia) indicated that both human and animal diseases causing organisms such as mosquitoes and bilharzia snails were occurred as the infestation detected (*Ingwani et al., 2010*). (*Opande et al., 2004*) also mentioned about its connection with human disease and the interviewed people answered that there was a general increase in disease as a result of the condition created by the weed. Various types of disease were reported such as skin rash, malaria, encephalitis, bilharzia and schistosomiasis. A similar consequence of the weed is also mentioned by (*D Tewabe, 2015*), that the weed has a potential of increasing malaria and schistosomiasis as it creats a better enviroment for mosquitos and other vectors.

2.4 Control measures

Water hyacinth is labeled as one of the most productive plant in the world. It brings various problems on the water bodies at which it appears and on the community around those areas. Therefore diverse controlling mechanisms such as physical removal, chemical methods (application of herbicides) and utilization of biological control agents has been applied to fight its infestation (*Malik, 2007*).

In general, the control methods can be classified among one of these three broad categories: physical (manual and mechanical removal), chemical (herbicidal), and biological control.

2.4.1 Manual removal and Mechanical control

This type of control measure is mostly implemented in developing countries. It can be performed by hand pulling or by using hand held tools such as pitch forks. However, it is very labor intensive and works for lower infestation levels (*M. Hill & Coetzee, 2011*). Zimbabwe was one of the African countries tried to use this control method in the early 1980s on Lake Chivero. Even if it was possible to remove 500 tons of the weed, the effect was knocked down by the aggressive regeneration of the weed over the lake (*M. P. Hill & Coetzee, 2008*). Ethiopia also took such kind of control strategy on Lake Tana from 2012 to 2014. Nevertheless, it could not decrease the continued growth from 20 ha in 2012 to more than 50,000 ha in 2014 (*Asmare, 2017*).

Mechanical controls can also be conducted using machineries which specifically are designed for aquatic weed control. The machineries might include bulldozers, boat, conveyors and dumb tracks (*M. P. Hill & Coetzee, 2008*). On the other hand, there are also two types of shredder boats to remove the weed through mechanical shredding (*Merry M. Mitan, 2019*). Zimbabwe also implemented mechanical control beside the manual one on Lake Chivero. Even though, 2 ha of water hyacinth was removed daily it couldn't bring a significant outcome in the control strategy. It was also implemented on Lake Victoria, but ended up with a limited success (*M. P. Hill & Coetzee, 2008*). In Ethiopia, a mechanical harvester was produced through collaboration of Mulat Industrial Engineering and Bahir Dar University. The machine had the capacity of harvesting 1000 m^3 per day, but it requires 4 liters of diesel/gas oil per hour (*bit.bdu.edu.et/HyacinthHarvesting, n.d.*). This method might not be recommended from economical point of view. In addition to that it is impossible to remove water hyacinth seeds through mechanical and manual control system which can stay dormant for 15 years (*Ingwani et al., 2010*).

2.4.2 Chemical control

This control method requires the application of chemicals such as herbicides (*Ingwani et al., 2010*). Chemical control has become famous since the end of World War II. After that, different types of herbicides are produced that vary in effectiveness and environmental consequence from one another. Among those, amitrol, 2,4-D amine, diquat, glyphosate and paraquat are well known around the world (*Téllez et al., 2008*). The application of chemical/herbicides is very simple compared to the mechanical one; the chemicals will be sprayed through backpack sprayers, helicopters or air-boats. Even if it demands lower labor force, it is more expensive than the physical method. Effectiveness of the control method is highly dependent on the size and application of water bodies i.e. it can be successful when it is applied in small (less than 1 hector) canals and impoundments (*M. Hill & Coetzee, 2011*).

The study of (*Agidie, Sahle, Admas, & Alebachew, 2017*) has suggested that it is also possible to use acetic acid in the control of water hyacinth. Application of 99% acetic acid showed similar result with 20% and 25% glyphosate that damaged the leaves of water hyacinth within 10 days. In South Africa, in the late 1970s, terbutryn was used to eradicate the infestation of water hyacinth on the Hartebeesport dam.

The main challenge of chemical control method is its potential of contaminating water bodies. Water hyacinth infestation, in many developing countries has occurred on water bodies which are being used for drinking water, fishing and washing. Therefore, spraying chemicals on these water bodies will contaminate it and might cause health problem on the community. On the other hand, the expense for skilled personnel make it very expensive for these countries (*M. P. Hill & Coetzee, 2008*).

2.4.3 Biological control

Both mechanical and chemical methods have a short-term effect in the fight against water hyacinth, but biological control is believed to be a long-term option to control the weed (*M. Hill & Coetzee, 2011*). Through this method, the aim is to reduce the infestation to an acceptable range through the application of living organism to utilize water hyacinth as their food source. These organisms should only reproduce on water hyacinth and should not attack other indigenous plant species (*Ingwani et al., 2010*).

Among the seven arthropod agents released worldwide, two weevils, Neochetina eichhorniae (Warner) and N. bruchi Hustache (Coleoptera: Curculionidae), are the most effective. N. bruchi and N. eichhorniae have been used to control water hyacinth in 30 and 27 countries respectively. In Uganda, 80 % of the weed biomass was eradicated after five years of impact by the two weevil species. The effectiveness of the weevils is also testified in Benin through the reduction of water hyacinth coverage from 100% to 5% in the period of eight years. The weevils also reduce the amount of water hyacinth by 90% on lake Edko which is found in Egypt and it only took them two years for the success (*Gebregiorgis, 2017*). On a study which is conducted at 30 sites in 8 districts bordering Lake Victoria, 142,000 weevils (mostly adult) were used to control the weed. After 26 – 36 months, the weevils had significant impact on the weed and ecological succession by other plant species, including hippograss, papyrus and morning glory take place (*Mailu, 2000*). (*Gebregiorgis, 2017*) has suggested utilization of N. bruchi to control water hyacinth under Ethiopian condition after comparing its population growth and percentage of success on plant damage with N. eichhorniae.

Other agents are also been considered since the time required through these arthropods to eradicate or reduce the infestation is perceived to be too long in some countries around the world (*M. Hill & Coetzee, 2011*).

2.4.3 Integrated control

The recommendation of experts for long term management of water hyacinth is using combination of control approaches instead of one control system. The achievement of long-term and sustainable solution can be gained through application of integrated approach which involves biological control agents (*Martínez Jiménez & Gómez Balandra, 2007*). There are number of ways to integrate existing methods, for example integration of pathogen, arthropods and chemical herbicides can be considered as combined control system (*Charudattan, 2010*).

Based on the study of (*Charudattan, 2010*) using control agents – Cercospora rodmanii, fungi pathogen and N. eichhorniae and N. bruchi, weevils (artthropods) - separately has low impact on removal of water hyacinth from Florida rivers. The arthropods were able to reduce shoot of hyacinth by 50%, whereas the pathogen reduced it only by 2%. On the other hand, combination of pathogen and arthropods resulted 99% control within 7 months. Another study which was conducted on Cruz Pintada reservoir also shows the effectiveness of integrated biocontrol. After 3 months of applying pathogens (Cercospora piaropia and A. zonatum) and insets (N. eichhorniae and N. bruchi), the reservoir became free of the weed (*Martínez Jiménez & Gómez Balandra, 2007*). The study of (*Gebregiorgis, 2017*) on the rift valley of Ethiopia found that, integration of plant pathogen (Alternaria alternata) and the two weevils resulted about 97% reduction of new ramets and 8% reduction of fresh weight.

On the other hand, control of water hyacinth through combination of pathogens and herbicides were also studied in outdoor pools. In this process, a pathogen (C. rodmanii) is applied first, followed by a herbicide (2,4,D amine-dimethylamine salt of 2-4-D-with concentration of 6.5%). The integrated control mechanism was able to damage 49% of the weed within 49 days after the pathogen application.

2.5 Utilization of water hyacinth

Recent control methods are only capable of keeping the infestation at an acceptable level. Despite the fact that water hyacinth is considered as one of the world's notorious aquatic weed, it can also have various uses. Its fibrous tissues and high energy and protein content make it attractive for a variety of useful applications despite high water content of more than 90%. Waste water treatment, compost, alcohol and biogas production can be mentioned as a potential of the weed (*Malik, 2007*).

2.5.1 Water hyacinth as phytoremediation

Its ability to grow in heavily polluted water together with its capacity for metal ion accumulation makes it suitable for wastewater treatment. Moreover, its infestation on natural wetland systems can be used as "nature's kidney" for proper sewage treatment to maintain water resources from pollution. It has been used to decontaminate inorganic nutrients, toxic metals and tenacious organic pollutants.

i. Removal of heavy metals

Pollutants (especially heavy metals) in wastewater will be absorbed by roots of water hyacinth and consequently the water quality will improve. The weed is well known for its effectiveness on removing mercury from effluent in which the root plays the major role in mercury uptake (Rezania, Ponraj, Talaiekhozani, et al., 2015). (*Liao & Chang, 2004*) studied the capacity of water hyacinth to absorb Cd, Zn, Pb, Cu and Ni in the Erh-Chung wet-lands, in Taiwan. Considering the production of water hyacinth to be 64 kg/m^2 (wet weight) in one growing season, the absorption capacity was 0.2 kg of Cd, 5.4 kg of Pb, 21.6 kg of Cu, 26.2 kg of Zn and 13.5 kg of Ni per hectare. In addition to that, on a laboratory study, the weed was able to survive in 1 up to 3 mg/l concentration of metal solution. Therefore, water hyacinth can remove acceptable quantities of heavy metals (Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn) from freshwater containing a mixture of those metals below 10 mg/l (*Soltan & Rashed, 2003*). According to (*Saleh, 2012*) it might also be possible to use hyacinth for radioactive waste solution (that contains ^{137}Cs and ^{60}Co) treatment. The maximum possible uptake was reached for both radioactive elements during 21 – 48 h from the initial time of treatment. It was possible to uptake almost 100 % of ^{60}Co from a solution when it exists separately. In contrast, the maximum removal of ^{137}Cs was achieved (which was 80%) in the presence of ^{60}Co and sunlight.

ii. *Removal of organic and inorganic compounds*

A large number of laboratorial studies have been conducted on water hyacinth to observe its potential for waste water treatment. In spite of its challenging nature to the world, its ability to uptake waste materials makes it more valuable on waste management process. Studies that are being carried out in laboratories and fields are concluding that the weed has exceptional quality on removing wastes from swine waste water (Rezania, Ponraj, Talaiekhozani, et al., 2015). Efficiency of water hyacinth in treating domestic waste water is measured in a laboratory study at the University Technology Malaysia. The result has showed that hyacinth was able to reduce the amount of phosphate (PO_4^{3-}) by 69.8%, ammonical nitrogen (NH_3) up to 95% and total organic carbon by 51.8 % while maintaining the pH in the acceptable range (5-6) (*Taylor, Rezania, Ponraj, & Din, 2014*). According to (*Kumari & Tripathi, 2014*), aeration and combining water hyacinth with Salvinia natans have a positive effect on removal of wastewater pollutants. The mixed culture with the help of aeration removed organic (BODs 84.5% and COD 83.2%) and inorganic (NO_3^--N 26.6 %, TKN 53% and PO_4^{3-}-P 56%) pollutants within 36 h of retention. On the other hand, the study also concluded that, in view of individual efficiency, water hyacinth has a better removal efficiency than S. natan. In a study that undertakes in Nigeria, it was possible to remove pollutants from textile, metallurgical and pharmaceutical wastewater through the application of water hyacinth. After 5 weeks of experiment the average removal of pollutants were find to be 53.03% of TSS, 64.41% of BOD, 65.4% of DO and 47.22% of nitrate-nitrogen (*Ajayi & Ogunbayo, 2012*).

2.5.2 Water hyacinth for production of organic fertilizers

Water hyacinth contains high concentration of plant nutrients such as N, P, K, Ca and Mg. Hence, the weed is being considered as a valuable resource to make compost and mulch. Different studies indicated that the plant leaves could store 15, 9, 13.9, 8.8 and 8.5 mg of Ca, K, Mg, P and N per gram of dry weight respectively. What makes it more desirable than other green manure is it can easily release nutrients. On the other hand, yield and protein content of several plants can be increased through combined utilization of different plant composts with water hyacinth (*Sanni & Adesina, 2012*).

(*Gajalakshmi & Abbasi, 2002*) studied the effect of water hyacinth compost/vermicompost on the growth of crossandra and several vegetables at the kitchen garden of five farmers in India. The soil that was amended with water hyacinth compost resulted in better height of crossandra plant, dense leaves, greater shoot:root ratio, better biomass per unit time and large length of inflorescence. The plants treated with vermicompost even showed a better result than the compost ones. In addition to that utilization of water hyacinth compost/vermicompost did not bring any adverse effect on the plants. The study of (*Sanni & Adesina, 2012*) also showed the impact of liquid water hyacinth fertilizer on the growth and yield of fluted pumpkin in Lagos, Nigeria. Fertilizer was applied after 5 weeks of planting and each plant was supplied with 16.7 kg of fertilizer. Nine (9) weeks after fertilizer application the yield was 16.08 kg which is greater than those without fertilizer, 12.42 kg. It also improved the quantity and quality of other plants.

2.5.3 Energy production from water hyacinth

The fuel crisis and tremendous effort to the alternative means of producing or utilizing energy opens the door to consider water hyacinth as one of a potential candidate. The aquatic weed can be a raw material for different kinds of products including bio-fuel, biomass and energy. Various researches have been done to produce biogas, ethanol and briquette from this notorious water plant (*Rezania, Ponraj, Din, et al., 2015*).

i. Biogas production

Aquatic plants such as water hyacinth could be exploited for biogas production due to the fact that it contains soft organic matter, 22 – 33.97 % hemicellulose by dry weight and very good C/N which is 20 – 35 (*Taylor, Jayaweera, Dilhani, Kularatne, & Suren, n.d.*). The conversion process takes place using anaerobic digestion technique in a reactor or digester to produce methane gas that can be used as a fuel for cooking, lighting or powering an engine to provide shaft work (Jafari, 2010).

Based on a research that is conducted in Kenya, it was possible to produce biogas from water hyacinth. The weed was mixed with cow dung in a ratio of 3:1 and the mixture mixed with water in a ratio of 1:1. After 32 days, while keeping the temperature between 22.8 – 36.6 ^{o}C, and pH 7.4 – 8.5, biogas of 49 – 53% methane, 30 – 33% carbon dioxide, 5 – 6% nitrogen and trace amount of hydrogen sulfide was produced (*Njogu, Kinyua, Muthoni, & Nemoto, 2015*). Other research that took place in Burkina Faso investigated the possibility of producing biogas from a mixture of water hyacinth and fresh rumen residues. It was possible to produce 151.4 m^3 of biogas within 65 days and 123.4 m^3 in 96 days during the dry, warm and dry, cold seasons respectively. It was also noted that for the same retention time, the total volume of biogas during the warm season is 1.8 times greater than that obtained during the cool season (*Almoustapha & Kenfack, 2009*). According to (*Case et al., 2013*) the amount of biogas produced from water hyacinth is dependent on the state of the plant. Average biogas production after 15 days of digestion were found to be 650 ml and 702 ml for fresh water hyacinth and dry water hyacinth respectively. The experiment also showed that 1 kg of water hyacinth yields 12.1 liters of biogas.

ii. Briquetting

The other option to control water hyacinth is through energy briquetting, which can utilize huge amount of the weed at a time. This kind of techniques can be a good input to a local community who are being challenged by increasing cost of energy (*Rodrigues et al., 2014*). In addition to that briquettes have number of advantages compared to the traditional energy sources (e.g. fire wood) such as greater heat intensity, cleanliness, convenience in use and relatively smaller space requirement for storage. Despite of its high moisture content, abundancy of water hyacinth facilitates its use as briquette (*Rezania, Ponraj, Din, et al., 2015*).

(*Davies, Davies, & Mohammed, 2013*) compared calorific value, fuel efficiency, water boiling time, ignition time and burning rate of water hyacinth briquette that was produced using plantain peel binder with mangrove wood, charcoal and firewood.

	WH briquette	Mangrove wood	Charcoal	Firewood
Calorific value (kcal/kg)	In the range	In the range	6552±4.73	4166±4.73
Fuel efficiency (%)	28.17±0.88	23.55±0.56	43.29±0.19	21.31±0.28
Ignition time (sec)	115±0.88	92.67±0.56	138.29±0.19	84.33±0.28
Burning rate (min)	1.25±0.016	2.05±0.016	0.97±0.016	2.49±0.016
Water Boiling time (min)	11.43±0.43	8.99±0.2	14.94±0.22	9.25±0.42

Table 2.1 comparison of characteristics of WH briquette with three traditional fuel sources: (in the range means between the value of charcoal and firewood).

According to (*Gunnarsson & Petersen, 2007*) it is also possible to use briquetting as a possible means of treatment. The weed was sun dried for few days, disintegrated, screened and chopped before briquetting. The treated hyacinth was compressed and resulted a material of an energy density of 8.3 GJ/m^3, which is close to charcoal that has 9.6 GJ/m^3.

iii. Ethanol production

Recently, researchers are giving attention for non-edible biomass such as lignocelluloses as raw materials to substitute first generation biomass resources - starch and sugar - for ethanol production. Utilization of aquatic weeds like as water hyacinth has tremendous advantages for water bodies and the local community and also it doesn't compete for arable land with food crops. Additionally, it also has low lignin content with acceptable amount of cellulose and hemicellulose (*Das et al., 2016*).

Production of ethanol from any lignocellulosic biomass comprises four fundamental steps, which are pretreatment, hydrolysis, fermentation and distillation. The pretreatment can be physical, chemical, biological or combination of these methods and the cost allocated for this process is a challenge for ethanol production process. The hydrolysis and fermentation of the pretreated biomass can be conducted separately (separate hydrolysis and fermentation (SHF)) or simultaneously (simultaneous saccharification and fermentation (SSF)). In both cases, hydrolysis of the treated biomass can take place by using chemicals such as sulfuric acid and hydrochloric acid or through the application of enzymes (e.g. cellulase). Finally, the produced alcohol will be enriched in the distillation column where a pure ethanol can be collected (*Sarkar, Ghosh, Bannerjee, & Aikat, 2012*).

Bioethanol production from $NaOH/H_2O_2$ pretreated water hyacinth was investigated by (*J. Yan et al., 2015*). The enzymatic hydrolysis (cellulose enzyme) produced 20.34 g/L glucose, 1.16 g/L xylose, 1.12 g/L cellobiose and 0.68 g/L of arabinose. In the SHF process, S.cervisiae angel converted glucose to ethanol with a maximum yield of 7.1 g/L while K. marxianus K123 achieved a maximum conversion of 6.41 g/L. in the SSF process, K. marxianus finished in 24 hrs with a maximum yield of 7.34 g/L, whereas S. cervisiae angel yeast achieved lesser yield of 4.45 g/L. The study of (*Idrees, Adnan, Sheikh, & Qureshi, 2013*), investigated the potential of water hyacinth for ethanol production through acid treatment. Among several acids presented in the study, H_2SO_4 treated water hyacinth gave maximum reducing sugar yield (0.327 g/g pretreated biomass) when hydrolyzed with cellulose enzyme. After fermentation, maximum ethanol concentration of 0.292% w/v was obtained and overall efficiency of the process was 59.3 %.

Several studies also showed that the potential of acid to hydrolyze lignocellulosic biomass for the production of ethanol. (*Masami, Usui, & Urano, 2008*) found 22.4 ml of ethanol/ kg of dried water hyacinth which was hydrolyzed by using 1% (v/v) sulfuric acid at 121 ^{o}C for 1 hour. The fermentation was conducted by an isolated yeast strain (candida intermedia) which was found from water bodies. Another study conducted by (*Reales-Alfaro, Trujillo-Daza, Arzuaga-Lindado, Castaño-Peláez, & Polo-Córdoba, 2013*) suggested that 2% (v/v) sulfuric acid could be optimum for hydrolyzation of water hyacinth. It was possible to obtain 33.3 g/L reducing sugar from the weed that was hydrolyzed with 2% H_2SO_4 at 121 ^{o}C for 20 minutes.

2.5.4 Other uses of water hyacinth

The water hyacinth has been found to be useful to produce some materials such as papers, animal fodder, yarn and rope, fiber boards and basket works on which researches are being conducted. In Indonesia, Philippines and India, water hyacinth is being used by small scale business groups to make papers. Production of rope from water hyacinth is similar to that of jute rope. Local furniture manufacturers in Bangladesh use the rope to add aesthetic value for their cane frame products. In addition to that, it is also possible to manufacture baskets for different purposes. In the Philippines and India people make baskets and mats for domestic use and the tourist industry. Animal fodder is another product of this invasive weed which can be used for ducks, pond fish and non-ruminant animals (e.g. pig). Farmers in china, boil size reduced hyacinth to with vegetable waste, rice bran, copra cake and salt to make a value add feed for their pigs (*Jafari, 2010*).

Water hyacinth handicraft was compared to papyrus handicraft at Lake Alaotra, in Madagascar. Mature papyrus has a height of around 3 meters whereas hyacinth is 1 meter at its maximum and also it needs a week to dry. Despite of these draw backs water hyacinth is getting acceptance in the community because of its strength, suitability for waving and better market price (3 times that of papyrus) (*Rakotoarisoa, Richter, Rakotondramanana, & Mantilla-Contreras, 2016*).

3. MATERIAL AND METHODS

3.1 Outline of the study

sample collection and preparation

- water hyacinth was collected from Koka Dam
- It was washed and disintegrated

characterisation of water hyacinth

- Moisture content is measured after drying in the convective oven at 105 ^oC.
- Thermal Gravimetric Analysis (TGA)
- XRD

Pretreatment

- The dried sample was ground (<2mm) and pretreated with a mixture of 1.5% H_2O_2 and 3% NaOH for 24 hrs in a shaker incubator at 150 rpm.

Characterisation of the pretreated sample

- TGA
- XRD

Hydrolysis of pretreated water hyacinth

- The pretreated sample was hydrolyzed using different sulfuric acid concentration

Factor	low	Medium	High
Sulfuric Acid conc. (%)	0.5	1	1.5
Temperature (^oC)	100	115	130
Time (min)	45	60	75

Quantifying the produced glucose

- by using UV spectrophotometer (wave length 315 nm)

3.2 Materials and chemicals

3.2.1 Equipment used for the production of glucose

The following list of equipment and Instruments were used to produce glucose from water hyacinth.

Table 3-1 list of equipment's and instruments

Instrument	Model	Application
Attrition mill	WRB 80 c/2 q	To reduce the size of water hyacinth
Laboratory test sieve		To separate samples based on size
Plastic bag	Falcon	To store samples
Convective drying oven	Memmert 100 - 800	To dry samples
Filtering crucible		As a filter media
Vacuum filter		To filter the hydrolyzed samples
Filter paper		As a filtering media
pH meter	Jenway 3505	To measure pH
Desiccator		To cool samples without absorbing moisture
Analytical Balance	FA 2004	To weigh samples
Measuring cylinder		To measure volume
Beakers		To prepare chemicals in different concentration
Conical flasks		To hold samples in the autoclave and shaker incubator
Vertical Autoclave		To hydrolyze water hyacinth
Centrifuge	Universal 320 R	To separate soluble and non-soluble part of the hydrolysate.
Shaker		To mix the sample at a specific rpm
Digital reading pipette		To measure small volume
Vortex mixer		To mix sulfuric acid and the sample

VIS-UV Spectrophotometer	UVD-3200	To measure glucose concentration in the hydrolyzed sample
X-ray diffractometer	D8 Avance Brucker	To observe the crystallinity of cellulose on the treated and raw water hyacinth

3.2.2 Chemicals and reagents

The following are list of chemicals and reagents which were used for the experimental section.

Table 3-2 list of chemicals and reagents

Chemicals and Reagents	Application
Water Hyacinth	As raw material
Distilled Water	Used for the whole process
Sodium Hydroxide	To delignify water hyacinth
Peroxide	To delignify water hyacinth
Sulfuric Acid	To hydrolyze the pretreated water hyacinth

3.3 procedures for glucose production

This study is conducted to observe the potential of water hyacinth (emboch) to produce glucose. Water hyacinth samples were taken from koka dam which is located at misraq Shewa zone of the Oromia region, close to Addis Ababa. The samples were brought to the chemical engineering laboratory which is found in Addis Ababa institute of technology to apply different kind of physical and chemical conversion. In this section the physical and chemical conversion that took place on the water hyacinth are discussed step by step. The main procedures for this study were

1. Preparing the sample
2. Pretreating the prepared sample
3. Production of glucose through hydrolysis of the pretreated sample
4. Filtration and centrifugation of the product

Based on these procedures, the whole production techniques were described in the following sections.

3.3.1 Sample preparation

The samples that were collected from koka dam had to be prepared before taking any further action. Therefore, the samples were washed using tap water to remove various impurities and dirt from the surface of the sample's leaves, stems and roots. The size of the samples (from root to the top leaf) was approximately between 50 cm – 1 m. This measurement was taken using a tape meter. Surface moisture and the moisture that was entrapped in the water hyacinth is removed by drying the sample using sunlight. It took 5 days to dry the whole plant, but it only took 2 and half days when the root, stem and leaf were separated. Since the size of the samples were very large to directly send it to the next process, size reduction took place to bring them to the available size range by using the centrifugal mill with a 2mm sieve in the mechanical unit operation laboratory in the school of chemical and bio-engineering.

Since the machine doesn't have 100 % efficiency there should be a sieving step to separate the coarse particles from the desired ones. Separation of samples was conducted by using vibratory feeders, which holds a series of sieve in a descending order 2 mm, 1mm, 710 µm, 500 µm, 355 µm, 250 µm and pan.

3.3.2 Characterization of water hyacinth

In this section the characteristics of water hyacinth was studied by using different techniques. Moisture content analysis, Thermo Gravimetric Analysis (TGA), and X-ray Diffraction were carried out to determine the moisture content of untreated water hyacinth, chemical composition and crystallinity of untreated and treated water hyacinth respectively.

1. *Moisture content analysis*

The moisture of the sample was measured using the surface moisture analyzer - (a product of Switzerland) which is found in the thermal and mass transfer laboratory - as soon as the sample were brought to AAiT. Although, there was some amount of moisture loss, I managed to measure the approximate moisture content. The fresh sample was measured and dried in a convection oven at a temperature of 105 ^{o}C. Then the sample was measured every 30 minutes until constant weight was recorded.

M_1 = mass of sample before drying

M_2 = mass of sample after drying

Moisture content (%) = $(M_2 - M_1)/M_1 * 100$ %..3.1

2. *Thermo Gravimetric Analysis (TGA)*

Thermal gravimetric analysis (TGA) was used to determine the chemical composition (cellulose, hemicellulose, lignin and the inorganic ash content) of the water hyacinth plant used in this study. 6.87 mg of dried water hyacinth was pyrolyzed in a nitrogen atmosphere - created by feeding nitrogen gas at a rate of 100 ml/min - in temperature range of 24 – 750 ^oC, with increasing rate of 10 ^oC /min. The generated weight loss vs temperature curve was then examined to determine the water hyacinth's chemical compounds based on the degradation of individual compounds as specified temperature range (*Harun, Dayang Radiah, Zainal Abidin, & Yunus, 2011*). The experiment is conducted at leather institute using TG instrument (SDT Q600 V20.9 Build 20).

3. *X-ray Diffraction (XRD)*

This test was performed to determine the crystallinity of untreated and pretreated water hyacinth. X-ray diffraction (XRD) patterns was recorded with D8 Avance XRD of Bucker powder diffractometer with Cu-Kα (λ=1.540593 – 1.544414 Å, 40 KV and 15 mA) radiation having a continuous scanning mode with speed of 10 deg/min. x-ray diffractogram of untreated and pretreated water hyacinth were obtained for 2θ angles ranging from 10^o to 60^o with scan step 0.02^o. Crystallinity of cellulose was calculated according to empirical method for cellulose as

CrI (%) = $[(I_{002} – I_{18.0^o})/ I_{002}] * 100$…………………………...3.2

Where CrI is crystallinity index, I_{002} is maximum intensity of lattice diffraction (002), and $I_{18.0^o}$ is intensity diffraction at 18.0^o, 2θ degrees. This XRD analysis was performed in college of natural science, department of chemistry, AAU.

3.3.3 Pretreatment of water hyacinth leaves and stems

This step is crucial to make the cellulose of the material easily available for the acid hydrolysis process. It reduces the amount of lignin present in the material and decreases the crystallinity of the cellulose. In this study the pretreatment was conducted with sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2). This experiment was conducted based on the study of (*J. Yan et al., 2015*). The samples that were dried and ground (which are less than 2 mm) were taken to conical flasks where the delignification process took place. A solution of 250 ml was prepared which comprised 3 % sodium hydroxide and 1.5 % hydrogen peroxide. The ratio of the dried and ground water hyacinth to the abovementioned solution was fixed at 1:25 (1 gram dried water hyacinth : 25 ml of solution). The solution was added to the conical flask which the powder and placed in the shaker incubator. The rpm of the shaker was fixed at 150 rpm and the sample stayed in the shaker incubator for 24 hrs. In the next day the pretreated sample was taken out of the incubator and filtered by a 32 µm sieve. Then the sample was washed by tap water to bring the pH value at 7. The final procedure in the pretreatment process was drying the washed sample. Washed sample was placed in convection oven to vaporize all the moistures from the sample. Grinding also took place since the water hyacinth formed a sheet like structure.

3.3.4 Hydrolysis of the pretreated sample

The hydrolysis part is required to convert cellulose of water hyacinth to glucose. There are several hydrolyzation techniques to convert cellulose into glucose. Among those methods, this research was conducted based on acid hydrolysis.

First the pretreated sample was sieved and those which are below 2 mm were collected for hydrolysis. Then different concentration of sulfuric acid solutions was prepared by diluting 98 % sulfuric acid using distilled water. There were three different solutions, which were 0.5 %, 1 % and 1.5 % sulfuric acid solutions. Other parameters such as time and temperature also have effects on the process and considered as factors for this stage. The experiment was conducted in three different time spans and temperatures which were 45 min, 60 min, 75 min and 100 ^{o}C, 115 ^{o}C, 130 ^{o}C respectively.

The experiment was done in randomized manner using design expert 12 software. The following data are displayed based on central composite design on design expert.

Table 3-3 Randomized experiment preparation using central composite design

Run	Sulfuric acid conc. (%)	Temperature (°C)	Time (min)
1	0.159104	115	60
2	1	115	60
3	1	115	85.2269
4	1	115	60
5	0.5	100	45
6	1	115	60
7	1	115	34.7731
8	1	140.227	60
9	0.5	130	75
10	0.5	130	45
11	1.5	100	75
12	1.5	130	45
13	1	115	60
14	1	115	60
15	1	89.7731	60
16	1.8409	115	60
17	1.5	100	45
18	1.5	130	75
19	1	115	60
20	0.5	100	75

Based on the above combination of the three factors the whole experiment was conducted in the bio-chemical and process laboratory. The pretreated sample was mixed with the abovementioned acid solutions and hydrolyzed at different temperatures and time spans. Those experiments which required temperature more than 121 °C were conducted using the hydrolyzer that is found in the process laboratory. Finally, the hydrolyzed sample was filtered through vacuum filter by using filtering crucibles as a filtering media. The supernatant comprised different simple sugar, including glucose.

3.3.5 Glucose measurement

After separating the supernatant from the cake, the next step was determining how much glucose is produced during the hydrolysis stage. This experiment was conducted based on the following procedure.

First the insoluble parts of the hydrolysate were removed through centrifugation of the sample in a centrifuge separator. Through decantation the supernatant which was free of the insoluble part was collected. Before determining the absorbance of the hydrolysate in spectrophotometer there needed to be a standard curve to convert the absorbance into glucose concentration in the sample. The curve was plotted by preparing glucose samples at different concentration, 0.2, 0.4, 0.6, 0.8 and 1 mg/ml. Then, to measure the absorbance, A 1 ml of solution was rapidly mixed with 3 ml of concentrated sulfuric acid (98%) in a test tube and vortexed for 30 s. The temperature of the mixture raised rapidly within 10 – 15 s after the addition of sulfuric acid. The solution was cooled in refrigerator to bring it to room temperature. Finally, UV light absorption at 315 nm was read using UV spectrophotometer.

4. RESULT AND DISCUSSION

4.1 Moisture content of water hyacinth

The average moisture content of water hyacinth used for this experiment was found to be 91.97 % using two replications. Constant weight of samples was obtained after 1 and half hours. Since water hyacinth is a floating aquatic plant, the higher moisture content was expected.

Table 4 mass of water hyacinth during drying

Mass of water hyacinth (g)	22.9	8.2	1.9	1.9
	21.9	7.4	1.7	1.7
Time (min)	0	30	60	90

4.2 Determination of the water hyacinth composition

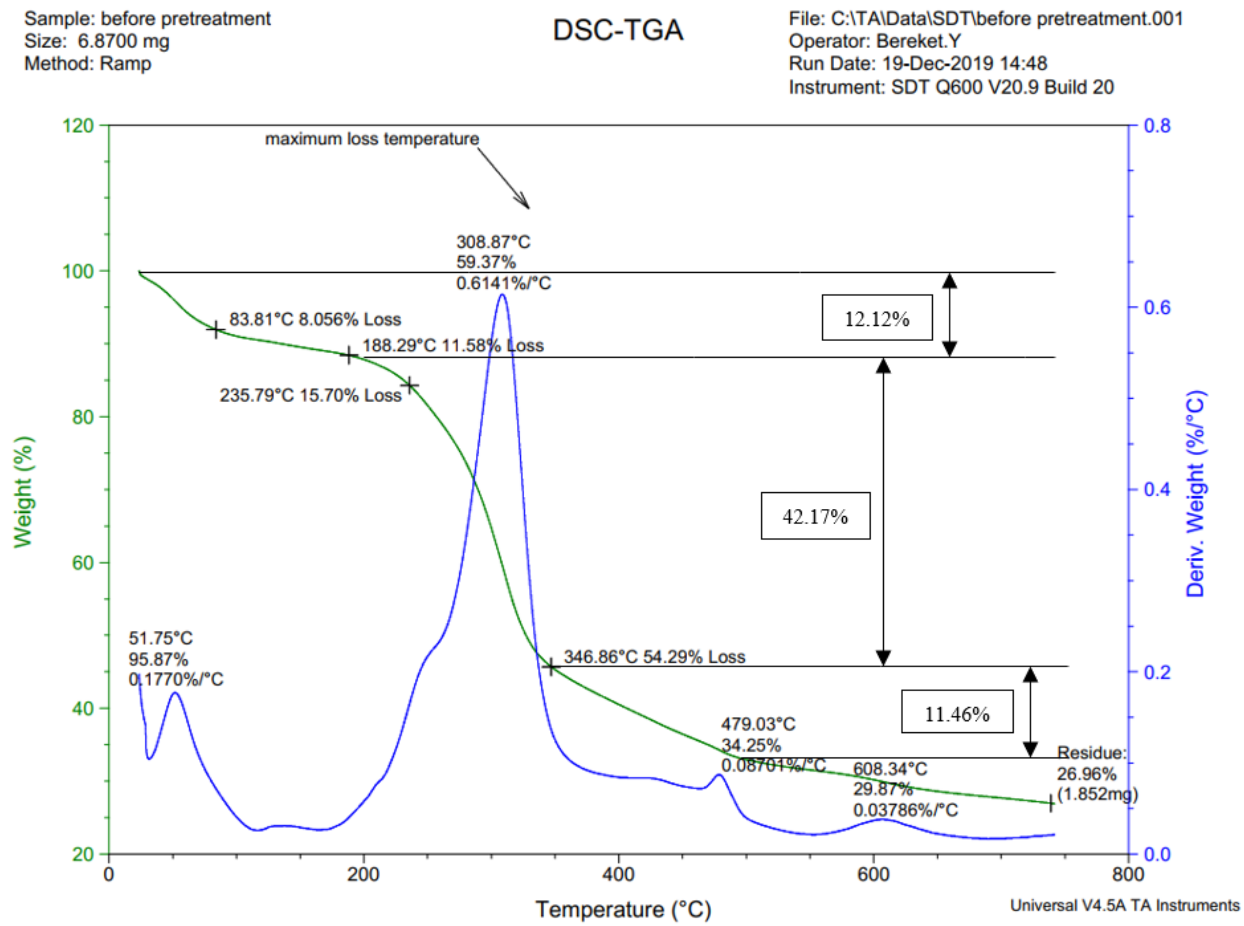

Figure 4 (A) Thermo Gravimetric (TG) and Differential Thermo Gravimetric (DTG) for untreated

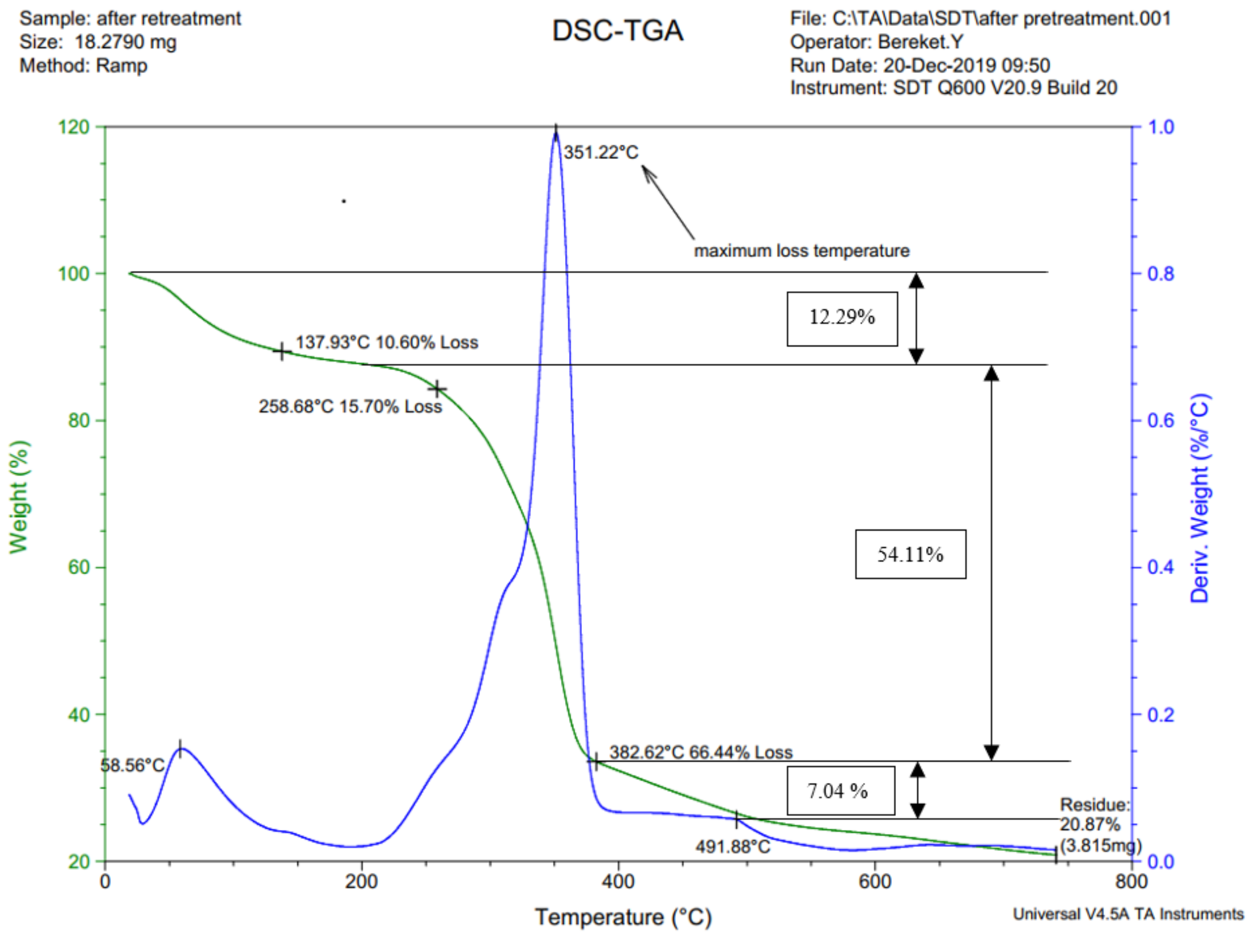

Figure 4 (B) Thermo Gravimetric (TG) and Differential Thermo Gravimetric (DTG) curves after pretreatment.

The result of a TGA of water hyacinth before and after pretreatment is given in Fig 4 A and B. Although there were several distinct weight loss stages on both curves, the discussion focused on the three critical stages. The first stage between 24 and 200 ^{o}C was attributed to evaporation of residual water and volatiles. In this stage the two samples lost approximately the same weight which were 12.12% for raw water hyacinth and 12.29% for the pretreated one. The second stage that was between 200 and 400 ^{o}C, was dedicated for cellulose and hemicellulose degradation. According to the graphs, there is a difference in cellulose and hemicellulose content of untreated and treated water hyacinth which were 42.17% and 54.11% respectively. On the final stage (400 – 500 ^{o}C), the amount of lignin was determined for both samples (11.46% before treatment and 7.04 after treatment). These two results not only show the composition of water hyacinth but also the effect of the pretreatment process. The residue (26.96% untreated and 20.87% treated) is non-combustible and is defined as the ash fraction of the water hyacinth.

Table 4-1 lignocellulosic component of water hyacinth

Component (% dry matter)			
Hemicellulose	Cellulose	Lignin	References
33.4	19.15	9.24	Gunnarson and Petersen (2007)
48.7	18.2	3.5	Nigam (2002)
27.1	19.7	NA[a]	Mishimat et al. (2008)
46.7[b]		27.7	Girisuta et al. (2008)
40.2[b]		6.5	Harun et al. (2011)
42.17[b]		11.46	This study

a: not available

b: the value represents total amount of hemicellulose and cellulose

4.3 XRD Analysis

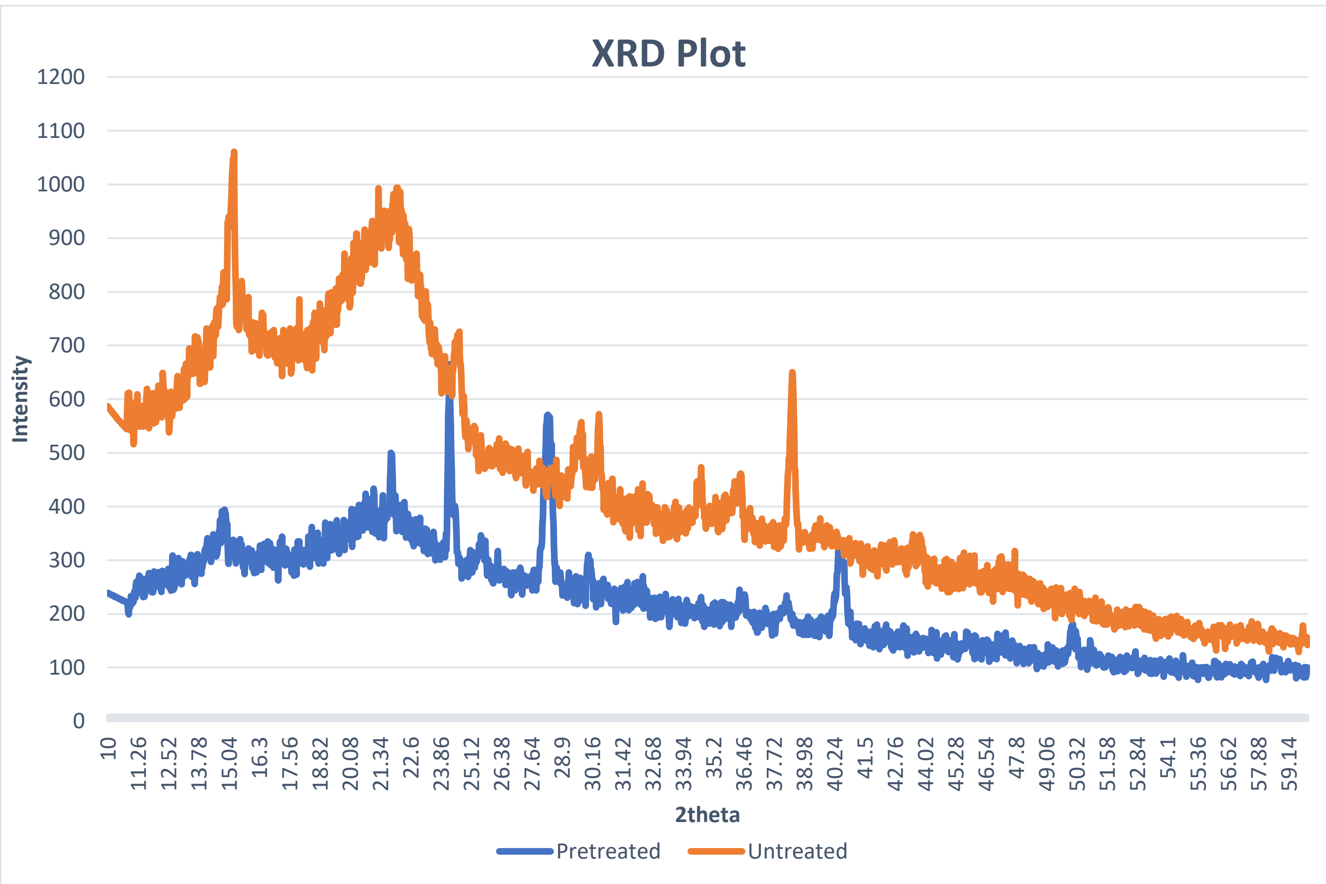

Figure 4.1 X-ray diffraction pattern of untreated and pretreated water hyacinth

XRD profile of untreated and pretreated water hyacinth (fig. 4.1) indicated that the crystallinity index of the untreated water hyacinth is 31.29% and that of NaOH/H_2O_2 pretreated sample is 53.68%. Then increase in crystallinity index of pretreated sample might be the result of removal of non-crystalline xylan and lignin from water hyacinth. As lignin removed and amorphous cellulose break down, the accessible surface area of cellulose increases, so does the crystallinity.

4.4 Hydrolysis performance of dilute sulfuric acid on water hyacinth under various hydrolysis parameters.

4.4.1 Analysis of glucose production

In this study, the production of glucose was investigated through dilute acid hydrolysis of water hyacinth. The amount of glucose in the hydrolysate was measured using UV-spectrophotometer. This device measured the absorbance of each sample at a wavelength of 315 nm and this absorbance value was converted into concentration using the standard curve. The absorbance of different glucose concentration that were prepared by dissolving dextrose in distilled water is provided on the Appendix.

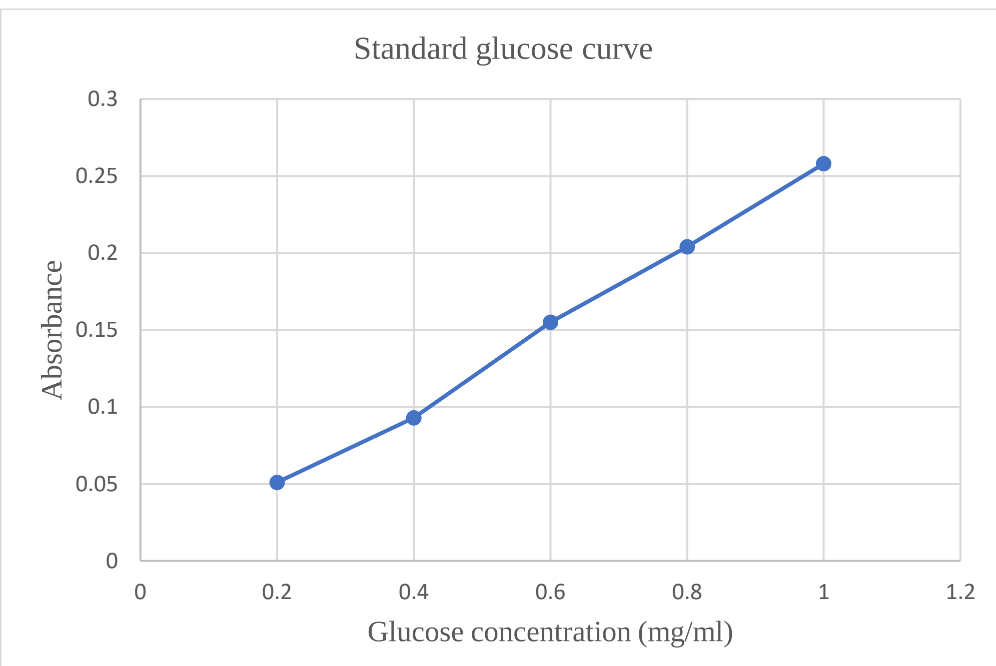

Figure 4.2 Calibration curve for the produced glucose

An equation that relates absorbance and glucose concentration was developed using the above plot. The equation became:

$$y = 0.257x - 0.0033 \qquad 4.1$$

The value of R^2 is 0.9974, which indicated that the equation was a best fit.

4.4.2 Statistical Analysis on hydrolysis performance

Optimization of the hydrolysis parameters was done using the pretreated water hyacinth sample. The optimization was carried out using central composite design with three factors (sulfuric acid concentration, hydrolysis temperature and hydrolysis time) and one response which was glucose concentration (mg/ml). Statistical analysis was carried out to determine correlation coefficients of the model as a function of the responses.

The sequential model sum of squares for glucose production is summarized in the following table. The models were selected because of their high correlation coefficient (actual and adjusted) values as shown in Appendix B.

Table 4-2 sequential model sum of square for glucose production

Sequential model sum of squares [Type 1]						
Source	**Sum of square**	**df**	**Mean square**	**F-value**	**P-value**	
Mean vs Total	1.80	1	1.80			
Linear vs Mean	0.0812	3	0.0271	3.09	0.0569	
2FI vs Linear	0.0282	3	0.0094	1.09	0.3878	
Quadratic vs 2FI	**0.1036**	**3**	**0.0345**	**41.18**	**< 0.0001**	**Suggested**
Cubic vs Quadratic	0.0078	4	0.0019	19.77	0.0013	Aliased
Residual	0.0006	6	0.0001			
Total	2.02	20	0.1008			

4.4.3 Test for significance and accuracy of the model

The levels of significance of the models were tested through the p-value. As shown on table 4.2, p-values less than 0.05 indicates the model terms are significant and p-values greater than 0.1 indicates the model terms are not significant.

In this case A, B, C, AB, AC, BC, A^2, B^2 and C^2 are significant model terms. The letters A, B and C represents sulfuric acid concentration, hydrolysis temperature and hydrolysis time respectively. Regarding this test, it can be noticed that varying all the three factors (acid concentration, hydrolysis temperature and hydrolysis time) have significant effect on the concentration of glucose in the solution. Furthermore, the interactions between these three factors have a significant effect on the hydrolysis process. However, hydrolysis time has a lower effect compared to the other two factors.

In addition to that the significance of the model was also supported through plotting the actual experimental values against the model predicted ones as shown in Appendix B. As it can be observed from the plot some of the actual data fit with the predicted ones and the others do not have a large variation. Therefore, the model is predictive.

The normal plot of residuals is also provided in the Appendix B. since the residuals lied approximately along on the straight line, the probability of facing problems with normality in the data is negligible.

Table 4-3 Analysis of variance for response surface quadratic model for glucose concentration

Response: concentration of glucose (mg/ml)

ANOVA for response surface Quadratic Model

Analysis of variance table [partial sum of squares – Type III

Source	**Sum of squares**	**df**	**Mean square**	**F value**	**p-value**	
Model	0.2131	9	0.0237	28.22	< 0.0001	Significant
A-Sulfuric Acid	0.0488	1	0.0488	58.18	< 0.0001	
B-Temperature	0.0276	1	0.0276	32.87	0.0002	

C-Time	0.0049	1	0.0049	5.82	0.0365	
AB	0.0125	1	0.0125	14.88	0.0032	
AC	0.0111	1	0.0111	13.23	0.0046	
4BC	0.0046	1	0.0046	5.49	0.0411	
A^2	0.0116	1	0.0116	13.87	0.0039	
B^2	0.0460	1	0.0460	54.88	< 0.0001	
C^2	0.0629	1	0.0629	74.98	< 0.0001	
Residual	0.0084	10	0.0008			
Lack of Fit	0.0078	5	0.0016	13.28	0.0065	Significant
Pure Error	0.0006	5	0.0001			
Cor Total	0.2215	19				

4.4.4 Development of regression model equation

The equations generated from the quadratic surface response for the hydrolysis of water hyacinth are shown on equation 4.1. The equation is mathematical representation of the experimental results and can be used to determine the hydrolyzing parameters required to achieve glucose production prior to the experiment. When regression coefficient has a positive value, increasing the associated factor also increases the response and a negative value would cause a decrease in the optimization parameters.

$$Glucose\ conc.\left(\frac{mg}{ml}\right) = 0.4027 - 0.0598\mathrm{A} + 0.0449\mathrm{B} + 0.0189\mathrm{C} - 0.0395\mathrm{AB} - 0.0373\mathrm{AC} - 0.0240\mathrm{BC} - 0.0284A^2 - 0.0565B^2 - 0.0661C^2 \quad 4.2$$

4.4.5 Effect of individual parameters on glucose production

Figures 4.3 – 4.5 show the effect of individual parameters on the hydrolysis of water hyacinth. It is observed that the amount of glucose produced varies as the concentration of sulfuric acid, hydrolyzing temperature and hydrolysis time differs.

The hydrolysis performance of water hyacinth is negatively affected by sulfuric acid. As the concentration of the acid increases the amount of glucose produced in the process decrease. The production of glucose is high at low acid concentration, but as the concentration started to rise glucose amount decreased almost in a linear fashion. At acid concentration of approximately

0.16%, glucose production was 0.379 mg/ml. A slight increase of the acid concentration also increased the amount of glucose in the hydrolysate. However, as the concentration increased above 0.5 it affects the hydrolysis process negatively. From figure 4.3 the amount of glucose became 0.415 mg/ml when the concentration was 1% and it further decreased to 0.267 mg/ml as the concentration kept rising and became approximately 1.84%.

As it can be observed from figure 4.4 the hydrolysis process is highly affected by the hydrolysis temperature. The concentration of glucose increases as the temperature rises until it reaches around 118 ^oC. As the temperature keeps increasing beyond this value, it affects glucose production negatively, so the production of glucose decreases as the temperature becomes higher.

The minimum temperature for this study was 89.7731 ^oC and the concentration of glucose at this temperature was 0.158 mg/ml. The second data is taken at the center point, which is at 115 ^oC and glucose amount has increased to 0.415 mg/ml. The temperature kept rising to detect if there is an increase on the glucose production. However, the amount of glucose in the hydrolyzate decreased and it has become 0.329 mg/ml, at 140.227 ^oC.

The effect of time on hydrolysis is depicted on figure 4.5. Hydrolysis time had significant effect on glucose production. Initially it is observed that time affects the process positively, but as it keeps increasing beyond the center point the amount of glucose started decreasing. The minimum amount of glucose detected was 0.198 mg/ml, which is found when the hydrolysis was conducted for 34.8 minutes. This amount kept increasing until the time span reached 60 minutes, which was 0.415 mg/ml. As the time span increased beyond this level the amount of glucose started decreasing and fall at 0.235 mg/ml when the time became 85.23 minutes.

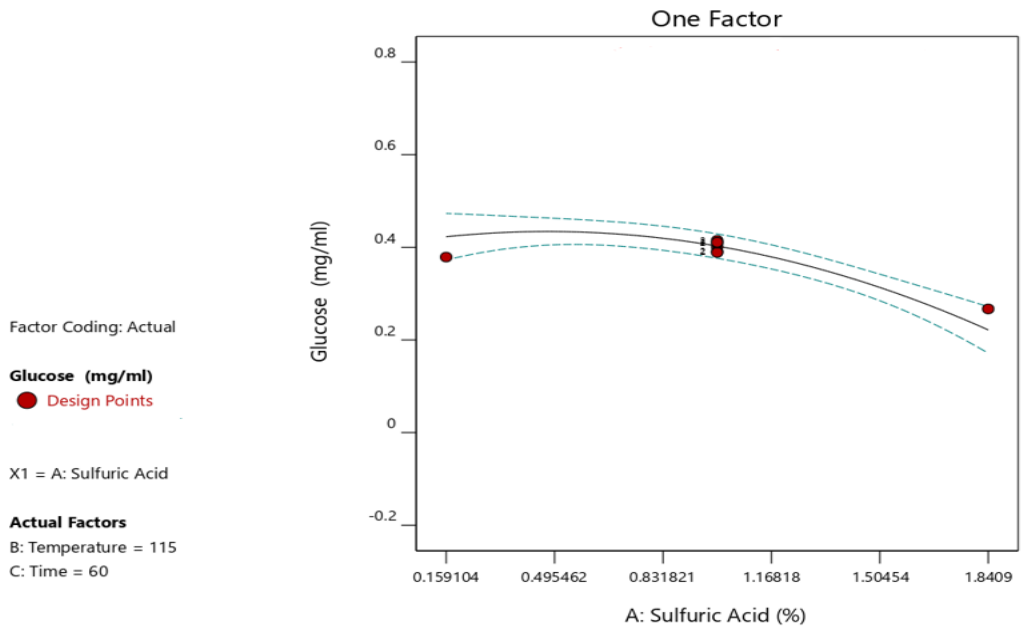

Figure 4.3 effect of sulfuric acid concentration when time and temperature are at the center point

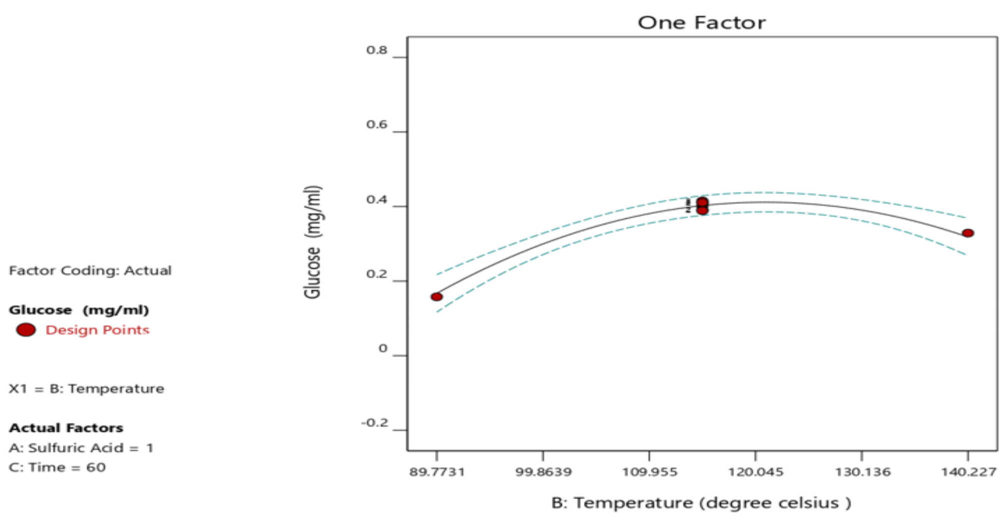

Figure 4.4 effect of temperature when time and sulfuric acid concentration are at the center point

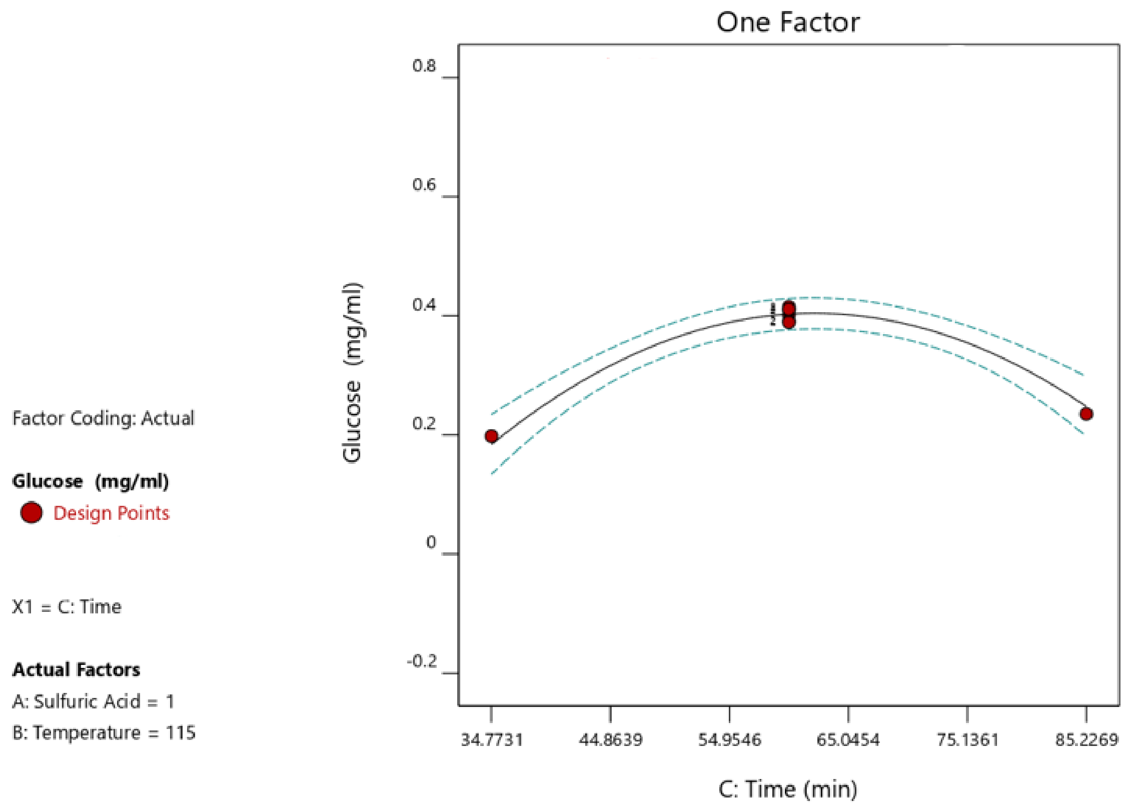

Figure 4.5 effect of time on hydrolysis when temperature and concentration of acid are at center point.

4.4.6 Effect of interaction between process variables

In this subtopic combined effect of the three variables, sulfuric acid concentration, temperature and time are discussed and illustrated on the 3D response surface plot (4.6 – 4.8). In addition to that, the contour plots are provided on Appendix B.

The interactive effect of hydrolysis temperature and sulfuric acid concentration on glucose production is depicted on figure 4.6. From the figure, a high glucose concentration was detected at low acid concentration and high temperature. The concentration of glucose in the hydrolysate started to fall when both temperature and acid concentration increased. In addition to that, decreasing the temperature while keeping the acid concentration at high and low percentage also decreased the amount of sugar produced during hydrolysis. On the other hand, a moderate increase on the acid concentration (approximately up to 0.5%) while increasing the temperature affected the process in a positive way. This condition is indicated in the dark red area, which has high glucose concentration with a maximum of slightly above 0.4 mg/ml.

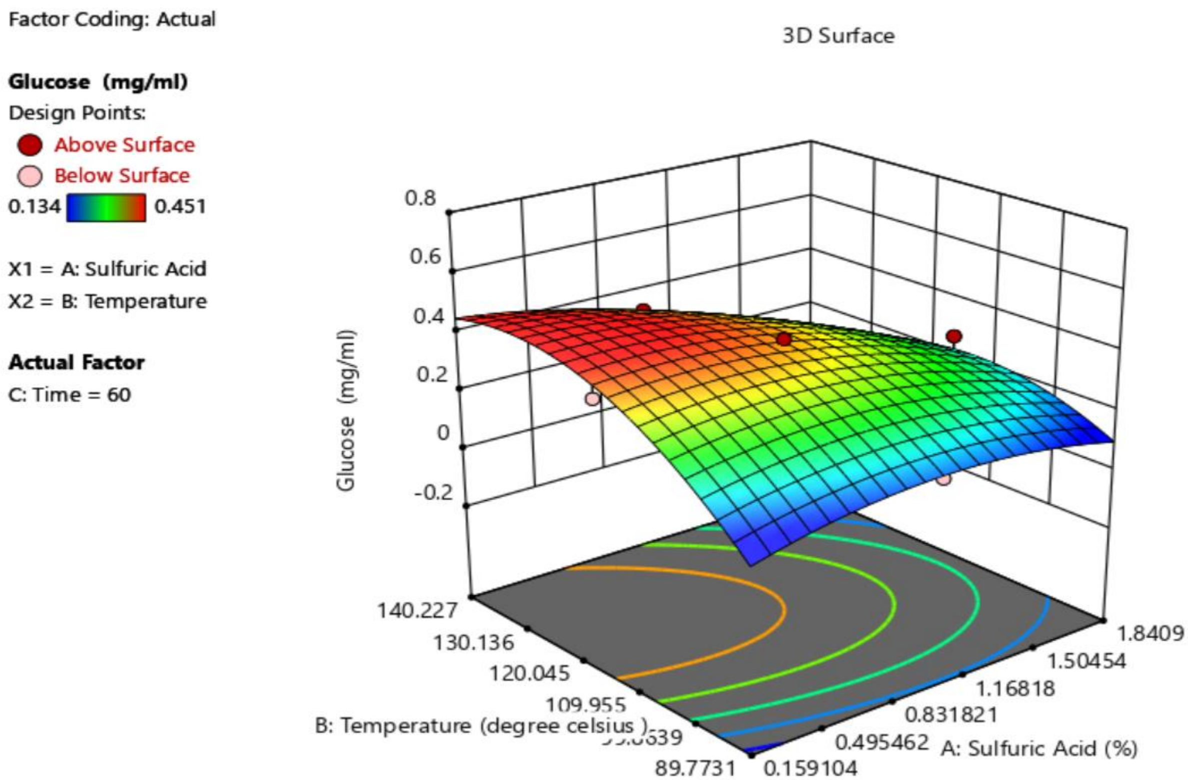

Figure 4.6 response surface plot of sulfuric acid concentration and temperature at 60 minutes hydrolysis time

The effect of interaction between time and sulfuric acid on the hydrolysis process is depicted on figure 4.7. The interaction between time and acid concentration had both negative and positive effect on glucose production. The amount of glucose produced at low time span and acid concentration was very low compared to the others. When both parameters started to rise so did the concentration of glucose. Nevertheless, increasing both factors beyond the center point (60 min, 1%) decreased glucose concentration in the hydrolysate. It is also observed from the 3D plot that keeping the acid concentration beyond the center point and increasing hydrolysis time reduces the simple sugar yield. This is also observed when the time was kept above the center point and the acid concentration kept rising. On the other hand, increasing the hydrolysis time slightly above 60 minutes while fixing the acid concentration around 0.5% increased glucose amount in the solution. Even if the fermentable sugar production facilitated by increasing the time when concentration of acid was 1%, increasing the time above the center point decreased the product.

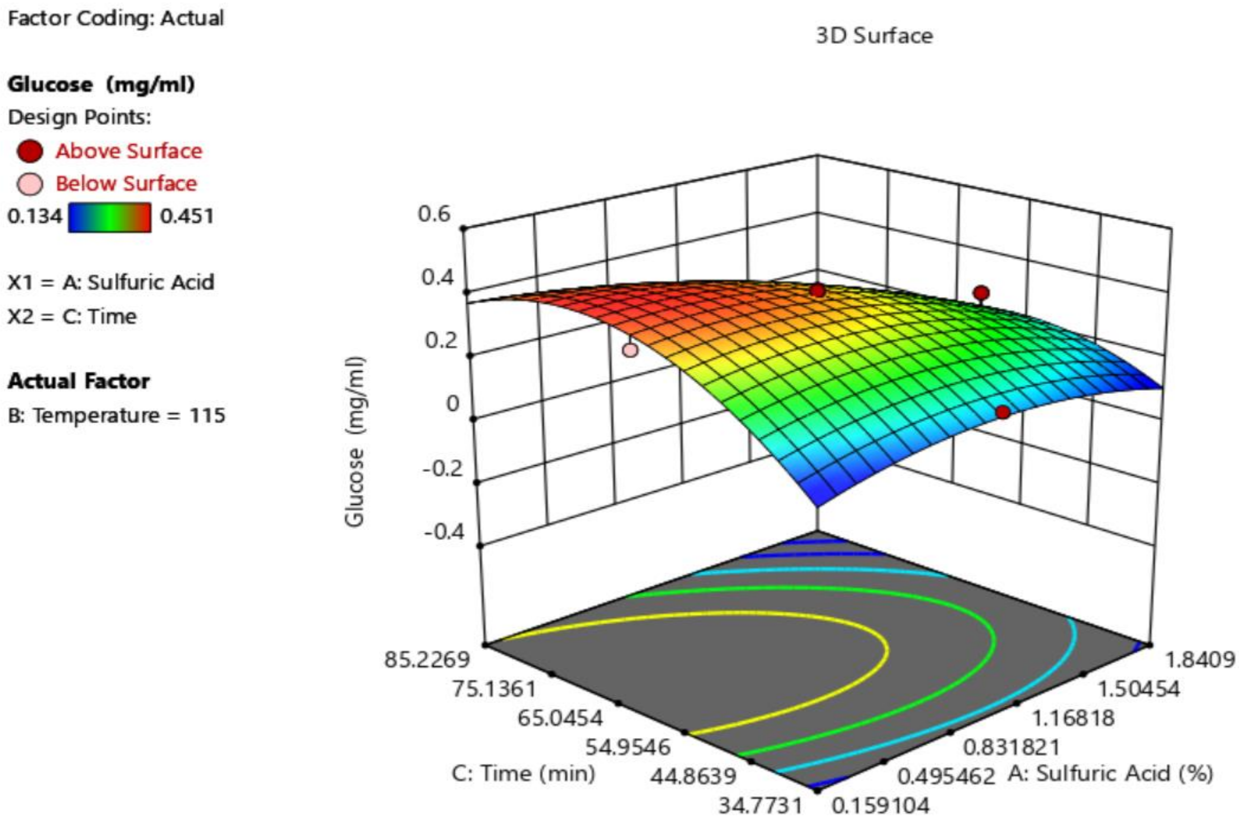

Figure 4.7 response surface plot of sulfuric acid concentration and time at 115 ^{o}C hydrolysis temperature

The effect of interaction of hydrolysis time and temperature on the hydrolysis process is depicted on figure 4.8. Production of glucose at lower hydrolysis time and temperature was almost impossible as it is illustrated on the plot. Both the temperature and time had similar effect on the process. As the both parameters kept increasing, the glucose concentration also started rising. The concentration of fermentable sugar in the hydrolysate increased from 0.167 to 0.369 mg/ml as the temperature raised from 100 to 130 ^{o}C while the time was kept at 45 minutes. Similarly, there was an increase of sugar from 0.167 to 0.332 mg/ml as the time increased from 45 to 75 minutes. A glucose yield of 0.451 was obtained when the temperature and time were increased from 100 ^{o}C and 45 minutes to 130 ^{o}C and 75 minutes, respectively. As it can be observed from the plot increasing both factors above this point had a negative effect on the yield of fermentable sugar.

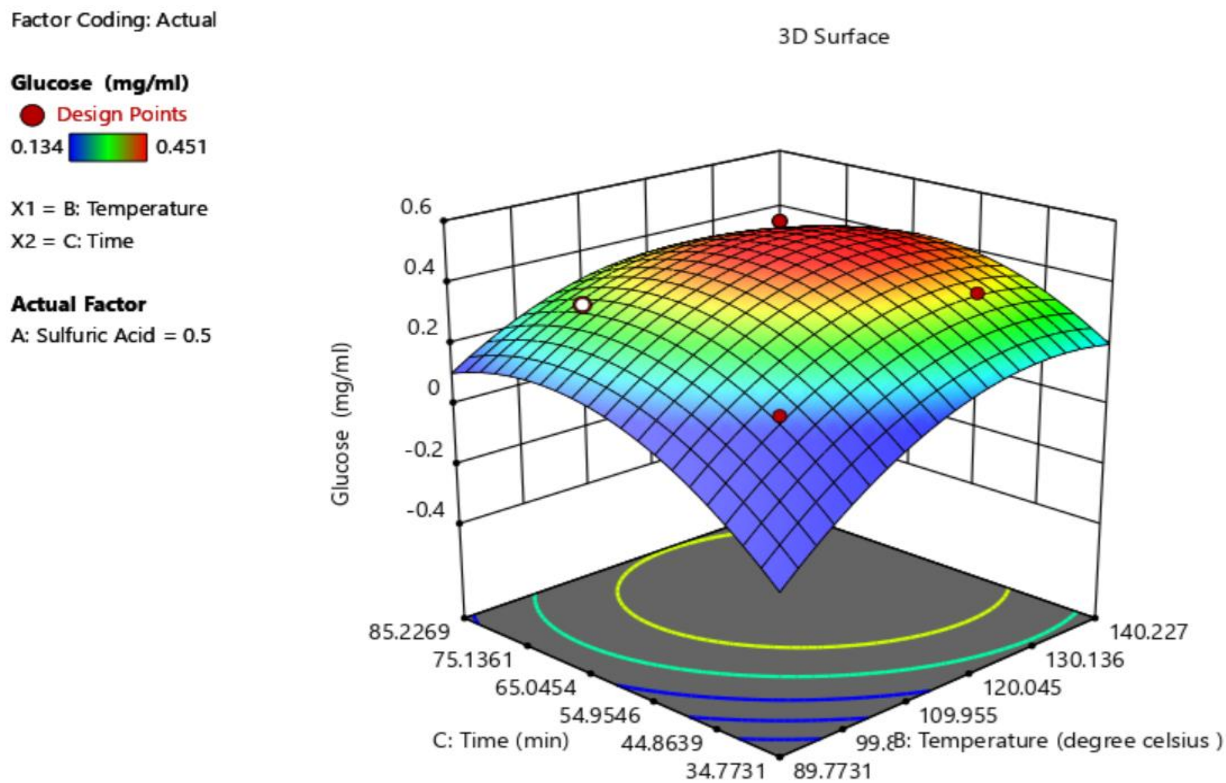

Figure 4.8 response surface plot of hydrolysis temperature and time at 0.5% acid concentration

4.4.7 Optimal hydrolysis parameters for glucose production

The hydrolysis parameters were optimized subject to constraint shown in Table 4-4. The objective was to maximize percentage of glucose in the hydrolysate. The hydrolysis process resulted 20 solution and the one with highest desirability was selected. The selected parameters were validated in the laboratory regarding suggested values as shown in Table 4-4. The results from the experiment confirmed the suggested ones.

Table 4-4 Optimization constraints for hydrolysis performance

Name	Goal	Lower limit	Upper limit
A: sulfuric acid conc (%)	Is in range	0.5	1.5
B: Temperature (^oC)	Is in range	100	130
C: Time (min)	Is in range	45	75
Glucose (mg/ml)	maximize	0.134	0.451

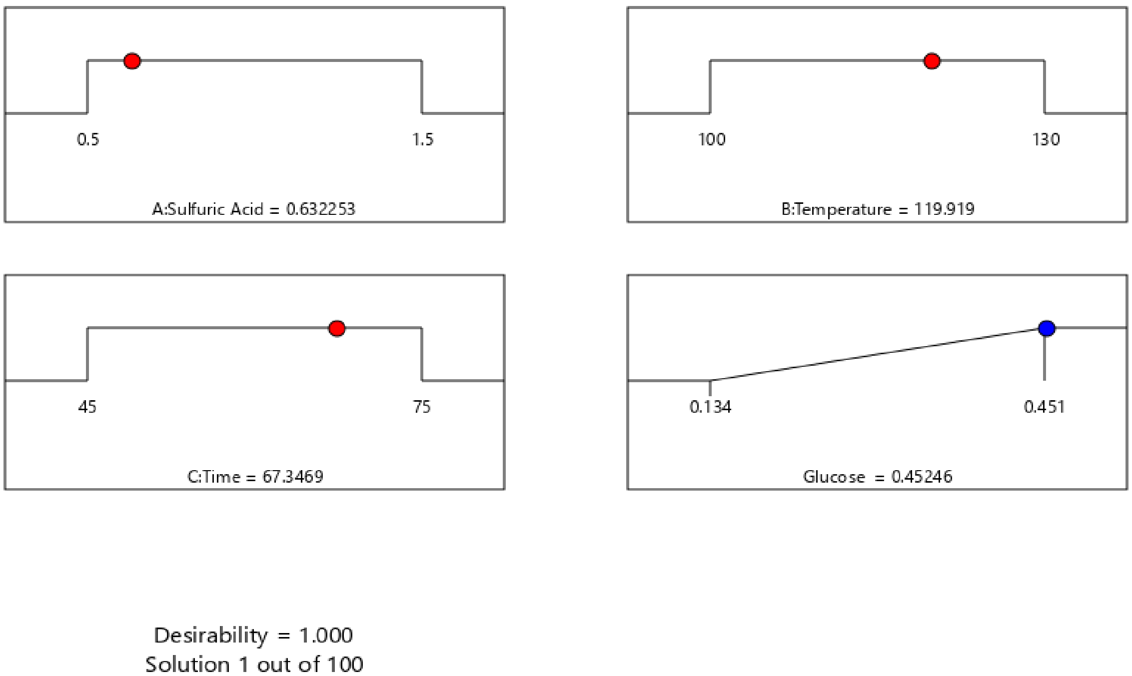

Figure 4.9 Optimum solution for glucose production

Table 4-5 Validation result

Parameter	Model predict	Experimental result
Acid concentration (%)	0.632253	0.5
Temperature (^oC)	119.919	130
Time (min)	67.3469	75
Glucose (mg/ml)	0.45246	0.451

5. CONCLUSIONS AND RECCOMENDATIONS

5.1 Conclusion

Water hyacinth has been observed as a potential biomass to serve as a low-cost feedstock to produce glucose that can be fermented to ethanol or changed to other products. Most of lignin present in water hyacinth can be removed by 3% NaOH and 1.5% H_2O_2 solution pretreatment at 4% (w/v) solid loading with a pretreatment temperature of 25 ^oC for 24 hours. This was observed through the increase of crystallinity index of water hyacinth 53.66% compared to 31.29% of the untreated sample. Furthermore, TGA also showed the effectiveness of the pretreatment stage. The untreated water hyacinth had 42.17 % (cellulose and hemicellulose) and 11.46% lignin, but after pretreatment it became 54.11% and 7.04% respectively.

A model for the hydrolysis of pretreated water hyacinth were performed through central composite design to optimize the parameters required for glucose production. The result presented herein provides clear evidence that hydrolysis time and temperature have strong effect on the hydrolysis process. The influence of acid concentration-in the given range-is low compared to the other factors. The effect of interaction of these parameters were also studied. The combined effect of temperature and time showed significant influence on the product and a high glucose yield was detected at 130 ^oC and 75 min. However, increasing these factors more than these values had a negative effect on the process. On the other hand, increasing the acid concentration resulted a slight decrease on the fermentable sugar production.

This model also provided the information about the interactive effect of the three parameters, sulfuric acid concentration, hydrolysis time and hydrolysis time on final concentration of glucose in the hydrolysate. The optimum point of the experiment was determined with respect to these three parameters and the optimum condition was found to be 0.5% sulfuric acid concentration, 130 ^oC hydrolysis temperature and 75 min which resulted 0.451 mg of glucose/ml. whereas, the model predicted the value to be 0.6323% H_2SO_4, 119.919 ^oC and 67.35 min for 0.4525 mg glucose yield.

Water hyacinth is well-known for its negative influence on the environment and society. However, in this study, its potential as a feedstock for glucose production has showed a considerable value. Therefore, it might also have a positive impact on the society besides its current treat as an invasive weed.

5.2 Recommendation

This paper tried to deal with the factors that have effects on the production of Glucose (fermentable sugar) from cellulosic biomass (water hyacinth) through acid hydrolysis. If further research is going to be done on this area, the following points should be taken into consideration.

- The hydrolyzing agent was selected based on literatures and availability of resources. Since current researches are dealing with enzymatic hydrolysis, it is better to compare both ways.
- There are various ways to pretreat lignocellulosic biomass. Therefore, it is also recommended to try other techniques other than the one investigated on this paper.
- There was solid residual after the hydrolysis part, further research can also be done on the utilization of this waste for other purposes.
- Characterization of water hyacinth also has a research potential to determine how much cellulose is available in the water hyacinth. Therefore, it is recommended to work on different characterization techniques, especially on those that can determine the composition of cellulose and hemicellulose separately.
- It is also recommended to perform preliminary design, economic feasibility study and establishing economic scale for fermentable sugar production from water hyacinth.

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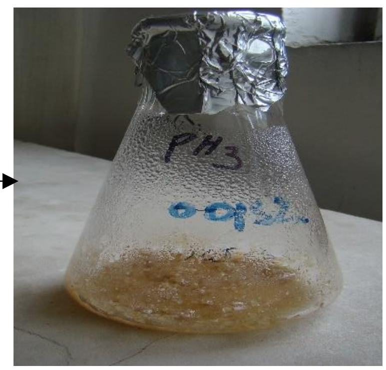
Appendices

Appendix A: Diagramatic illustration of the experiment

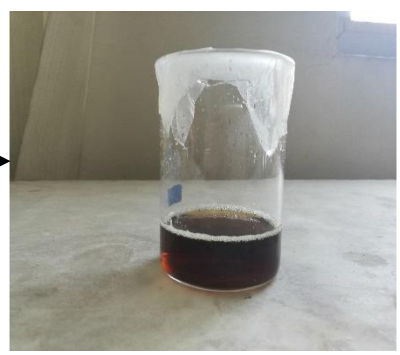


Raw water hyacinth drying size reduction





Pretreatment wash to neutralize

Centrifuge neutralisation

Appendix B: Experimental Design Outputs

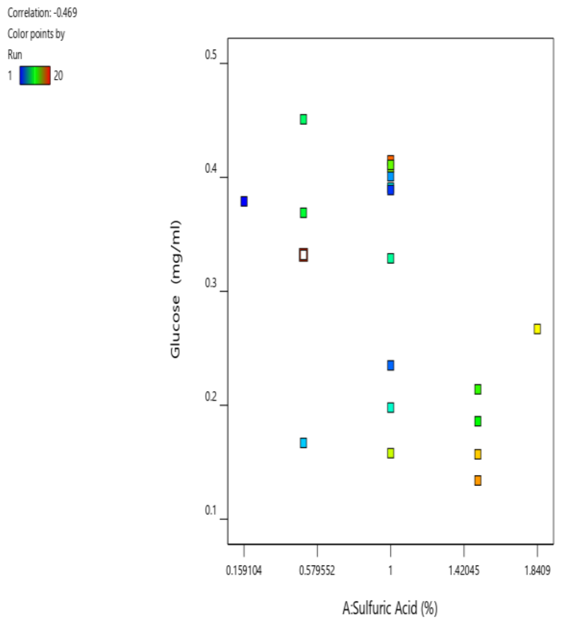
Table B-1 Design summary for hydrolysis process

File Version		12.0.3.0								
Study Type		Response Surface		**Subtype**	Randomized					
Design Type		Central Composite		**Runs**	20					
Design Model		Quadratic		**Blocks**	No Blocks					
factor	**name**	**units**	**Type**	**min**	**max**	**Coded low**	**Coded high**	**mean**	**Std. dev.**	
A	Sulfuric Acid	%	Numeric	0.1591	1.84	0.50	1.50	1.0000	0.4239	
B	Temperature	degree celsius	Numeric	89.77	140.23	100.00	130	115	12.7	
C	Time	min	Numeric	34.77	85.23	45.00	75	60	12.7	
Response	**Name**	**Units**	**Observations**	**Analysis**	**Minimum**	**Maximum**	**Mean**	**Std. Dev**	**Ratio**	**Model**
R1	Glucose	mg/ml	20	Polynomial	0.134	0.451	0.2996	0.1080	3.37	Quadratic

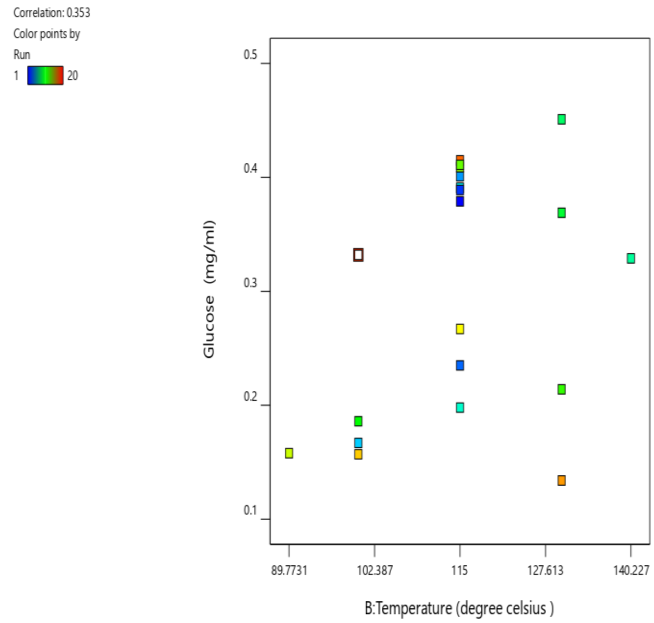
Table B-2 Absorbance and Glucose concentration on each run

Run	Sulfuric acid concentration (%)	Hydrolysis temperature (°C)	Hydrolysis Time (minutes)	Absorbance	Glucose concentration (mg/ml)
1	0.159104	115	60	0.094	0.379
2	1	115	60	0.097	0.389
3	1	115	85.2269	0.057	0.235
4	1	115	60	0.1	0.401
5	0.5	100	45	0.04	0.167
6	1	115	60	0.097	0.391
7	1	115	34.7731	0.048	0.198
8	1	140.227	60	0.081	0.329
9	0.5	130	75	0.113	0.451
10	0.5	130	45	0.092	0.369

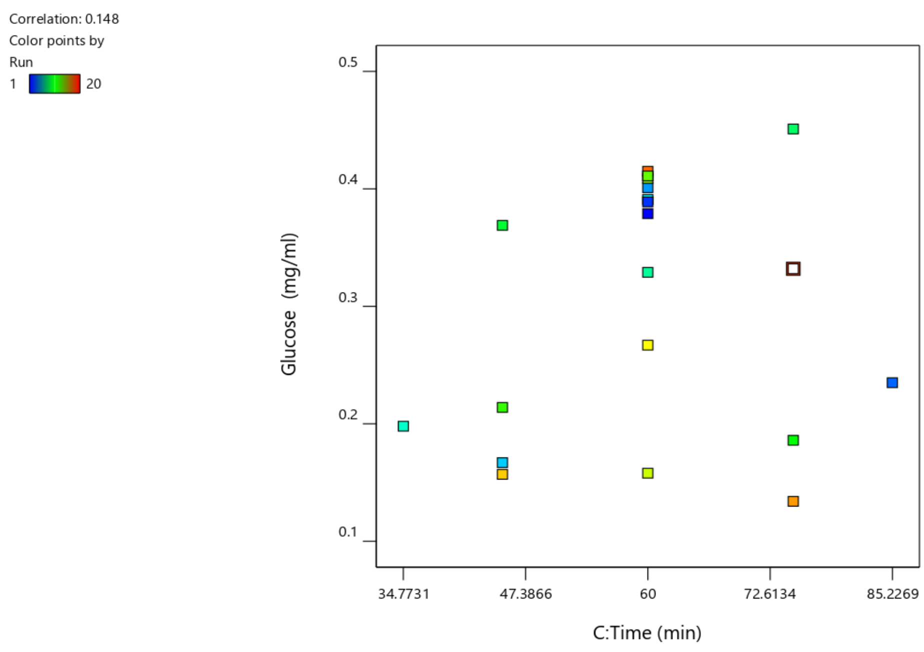
11	1.5	100	75	0.045	0.186
12	1.5	130	45	0.052	0.214
13	1	115	60	0.102	0.411
14	1	115	60	0.102	0.409
15	1	89.7731	60	0.037	0.158
16	1.8409	115	60	0.065	0.267
17	1.5	100	45	0.037	0.157
18	1.5	130	75	0.031	0.134
19	1	115	60	0.103	0.415
20	0.5	100	75	0.082	0.332



(A)



(B)



(C)

Figure B-1 Scatterplot of (A) sulfuric acid concentration, (B) temperature and (C) time

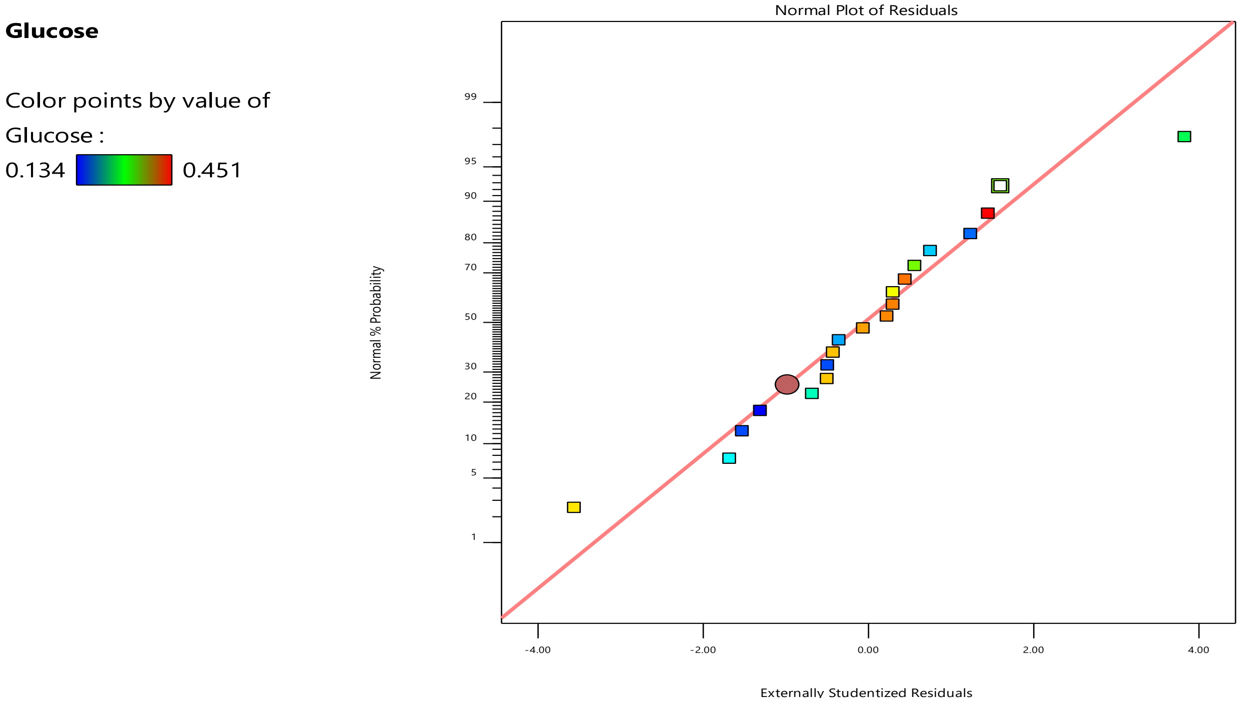


Figure B-2 Normal probability plot

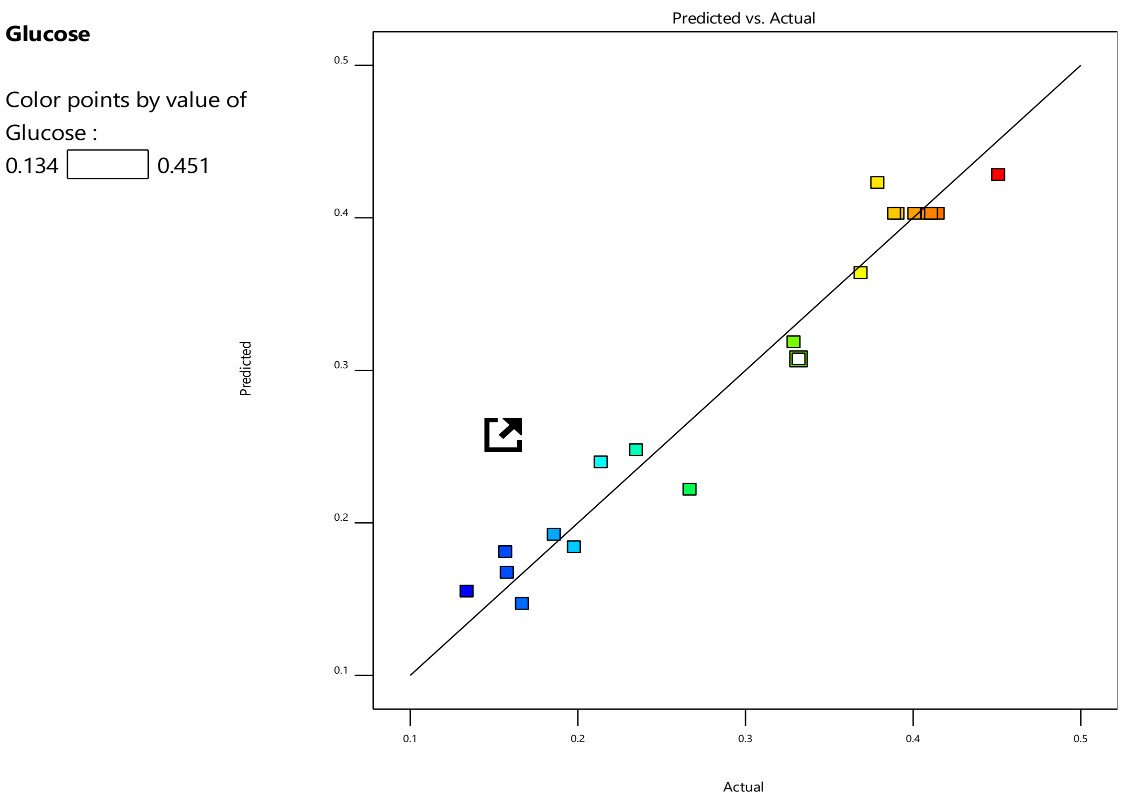
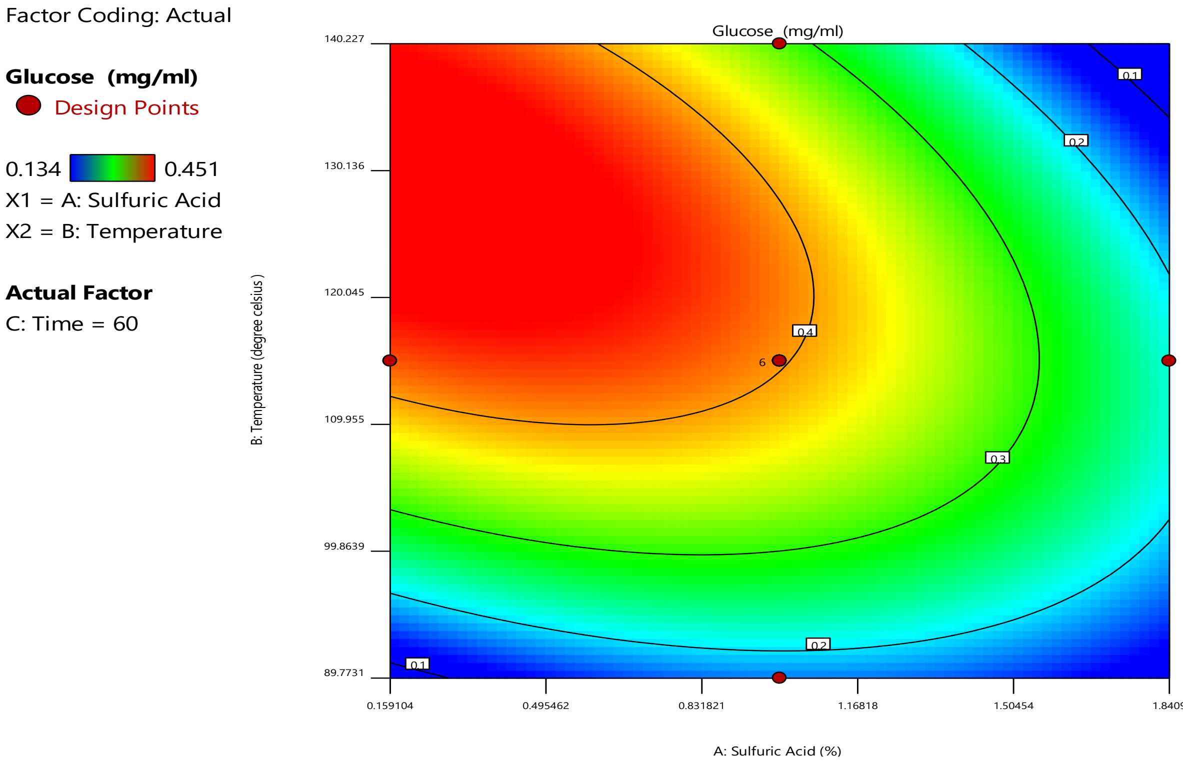
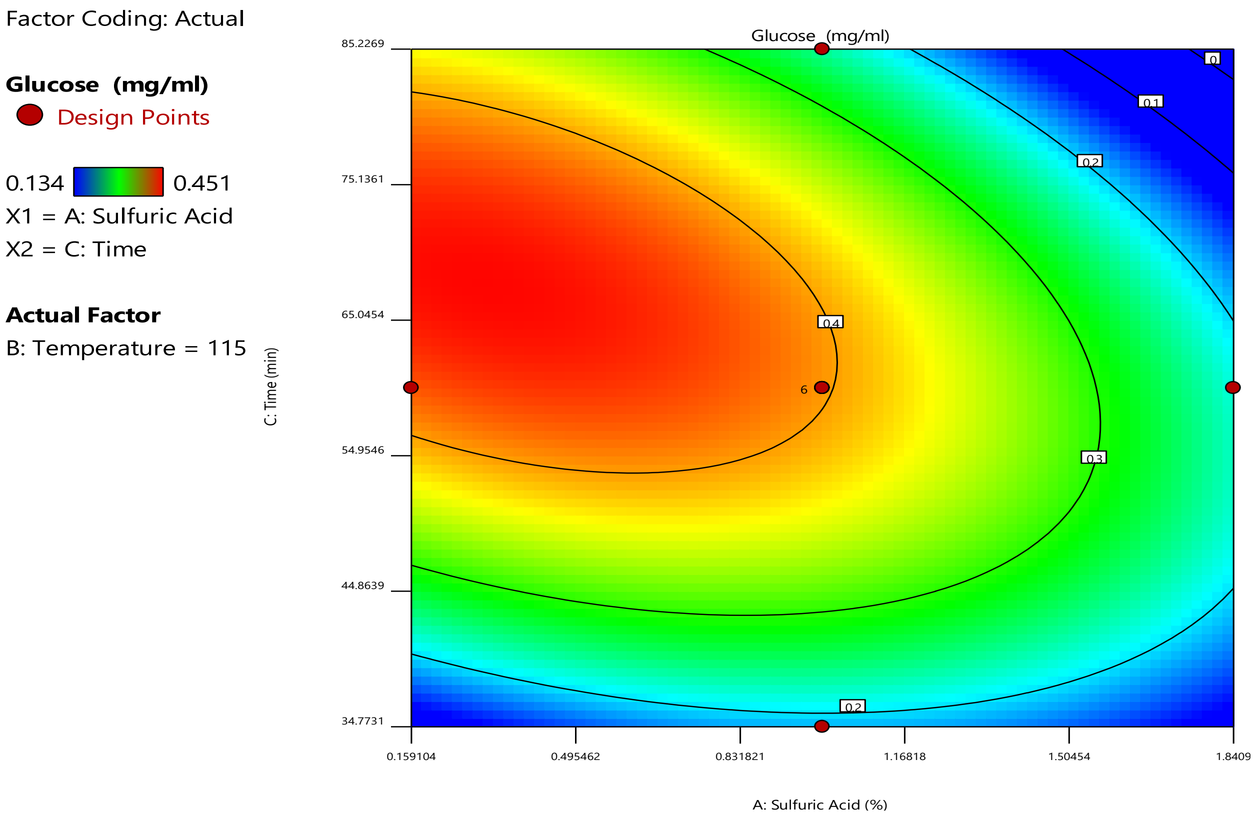


Figure B-3 Predicted Vs Actual plot



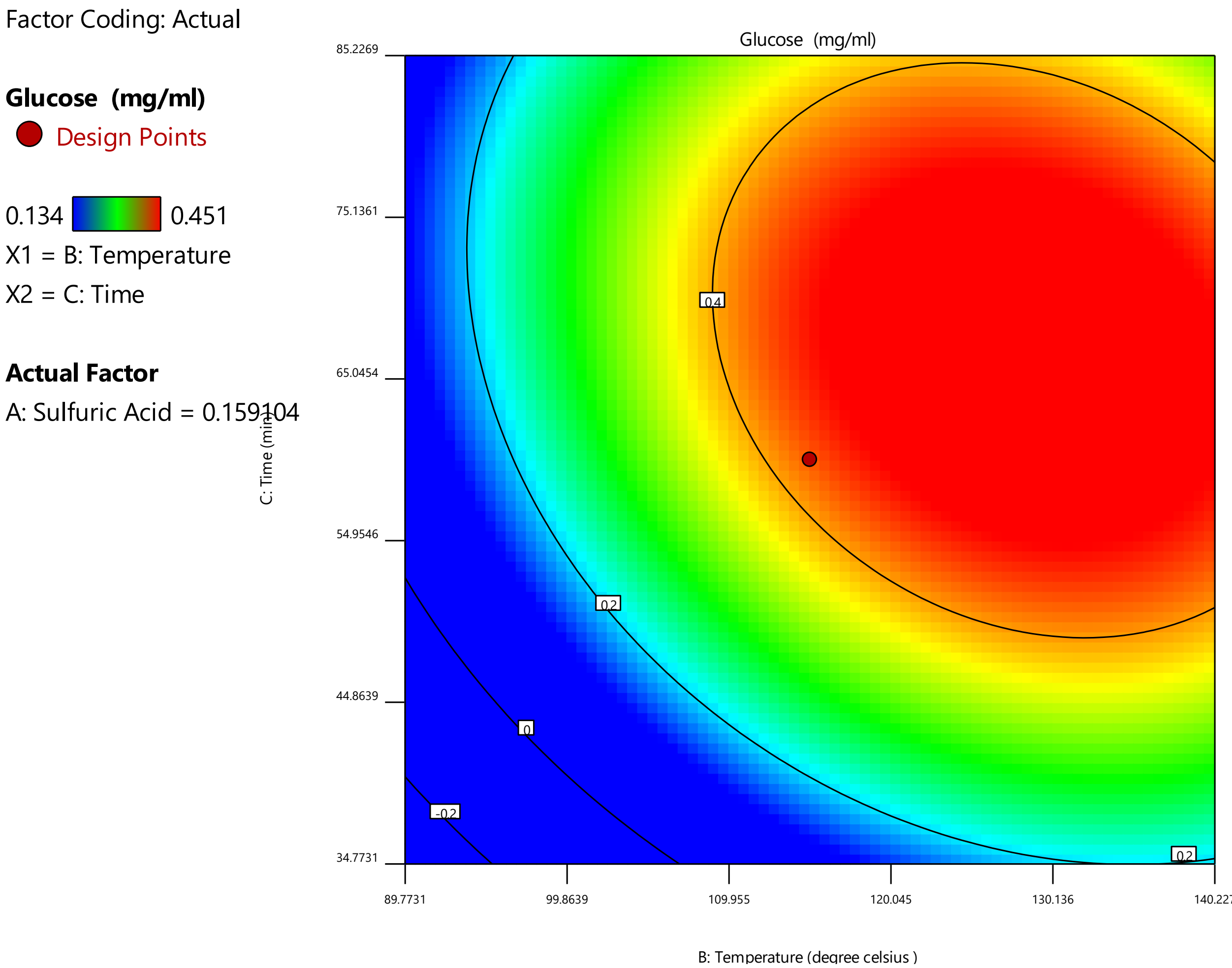
Factor Coding: Actual
Glucose (mg/ml)
Design Points
0.134
0.451
X1 = A: Sulfuric Acid
X2 = B: Temperature
Actual Factor
C: Time = 60
Glucose (mg/ml)
B: Temperature (degree celsius)
140.227
130.136
120.045
109.955
99.8639
89.7731
0.159104
0.495462
0.831821
1.16818
1.50454
1.8409
A: Sulfuric Acid (%)
0.1
0.2
0.3
0.4
6

(A)



Factor Coding: Actual
Glucose (mg/ml)
Design Points
0.134
0.451
X1 = A: Sulfuric Acid
X2 = C: Time
Actual Factor
B: Temperature = 115
Glucose (mg/ml)
C: Time (min)
85.2269
75.1361
65.0454
54.9546
44.8639
34.7731
0.159104
0.495462
0.831821
1.16818
1.50454
1.8409
A: Sulfuric Acid (%)
0
0.1
0.2
0.3
0.4
6

(B)



(C)

Figure B-4 Contour plots of (A) sulfuric acid and temperature at 60 min, (B) sulfuric acid and time at 115 ºC and (C) temperature and time at 0.159%